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A Record of the Progress of Pharmacy and the Allied Sciences

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Vol. 101 OCTOBER, 1929 No. 10

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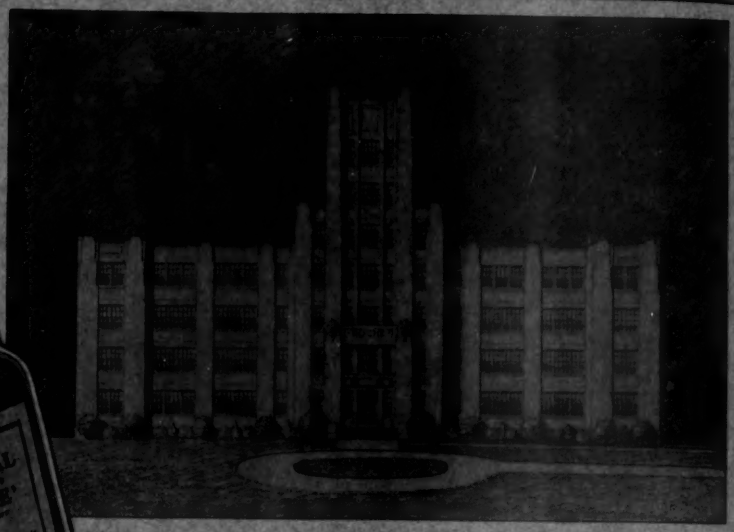

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NUTLEY, NEW JERSEY

THE AMERICAN
JOURNAL OF PHARMACY

VOL. 101 OCTOBER, 1929 NO. 10

SELECTED EDITORIALS

THE UNITED STATES PHARMACOPŒIA*

ON PAGE 989 OF THIS ISSUE, the Council on Pharmacy and Chemistry appeals to the organizations entitled to send delegates to the United States Pharmacopœial Convention to choose representatives noted for high ideals, breadth of vision, trained understanding and sound judgment. The Council's appeal is timely. Those whose duty it is to select and appoint delegates to represent their group or institution should without delay select persons fitted for the task and take steps to insure the presence of these delegates at the convention.

Although Federal laws concerned with the control of the purity of drugs provide for the enforcement of drug standards set forth by the Pharmacopœia, the book is not published by or under the control of the Federal Government. It is published by authority of the United States Pharmacopœial Convention. This body is composed of members who are sent as delegates by national or State associations of physicians, pharmacists and other groups concerned with medicine and drugs, by schools of medicine and of pharmacy, and by certain Government services. The convention meets once every ten years, and its chief function is the selection of the Committee of Revision of the United States Pharmacopœia. To this committee is assigned the task of making any desired changes in the Pharmacopœia then in force and of issuing a revised edition of the book. The next Pharmacopœial Convention has been called for May 13, 1930, at which time the delegates appointed by the constituent bodies will meet in Washington, D. C., and inaugurate the preparation of the next, the eleventh, revision of the United States Pharmacopœia.

Originally the revision of the Pharmacopœia was controlled exclusively by physicians, the Council explains, but as the pharmaceutic

*Reprinted from *Journ. Amer. Med. Assoc.*

problems increased in magnitude the pharmaceutic profession was taken into equal partnership. With the progressive evolution of scientific pharmacy, and especially with the introduction of more scientific standards and tests, the major share of the technical work of the revision fell on the pharmaceutic members, who therefore justly became numerically preponderant in the "Revision Committee." At that time—the period when instruction in medical schools in subjects related to therapy and drugs was wofully deficient, and when conditions made necessary the establishment by the American Medical Association of its Council on Pharmacy and Chemistry—the Pharmacopœia promised to degenerate into a mere book of standards for drug control officers. Then, in 1916, when the ninth revision of the Pharmacopœia had just made its appearance, *The Journal* (Sept. 2, 1916, p. 750) pointed out that the Pharmacopœia was a book of standards for drugs but not a book of standard remedies: that the book included substances which had been shown to be inert, complex and obsolete mixtures, and drugs which had been tried and found wanting; that it contained much matter of little interest to the physician and entirely foreign to scientific medicine. Largely as a result of the renewed interest in scientific drug therapy which was created by the reports of the Council on Pharmacy and Chemistry and the publications and teaching of its members as individuals, so much interest was taken in the following revision of the Pharmacopœia that medical members of the revision committee were in effect put in charge of the scope of the book at the convention held in 1920. They were delegated to decide which of the drugs in the ninth revision were to be retained in the tenth and which were to be omitted as being of insufficient usefulness.

With the principle of additions and deletions thus secured, and with the question of additions made relatively simple by the probationary term which new drugs serve in New and Nonofficial Remedies, the tenth revision of the Pharmacopœia became a book with which both physicians and pharmacists may justly be satisfied. In order that the next revision may correctly reflect the advances achieved in drug therapy during the last ten years, the medical and other bodies entitled to send delegates to the coming convention should give serious consideration to the appeal of the Council on Pharmacy and Chemistry. Those who are sent as delegates should have the sane broad-mindedness necessary for carrying on the constructive policies that have made for the success and usefulness of the last revision of the Pharmacopœia.

WISDOM AND AUTHORITY SHOULD BENEFIT ALL*

IN SOME RESPECTS mankind has progressed very slowly since the days of the caveman. The amazing amount of credulity and superstition that still lingers shows how hard it is for the average person to shake off old ideas and imaginings. The immense number of quacks who prey upon this unfortunate phase of millions of minds illustrates more vividly than words the extent of ancient beliefs and ignorance which exists in these modern days of a civilisation that has produced many marvellous things.

A well-known scientist once mentioned to the writer that our possession of mentality was probably the greatest mystery of human life. We know that the brain is a wonderful recording machine, and, for the most part, the impressions made upon this marvellous instrument are responsible for much of our ideas and thoughts. But where do imagination and inspiration come from? What power or influence is responsible for the gifted artists and musicians and all the other geniuses who appear regularly among the human race? We might also ask whence comes the selfishness of the learned among us, who know, yet withhold, things which are necessary for the preservation of health in the individual or his welfare generally. Why is it that the medical profession, knowing full well the menace that quacks are to the public at large, are still calmly surveying the dangerous situation without protest?

Then, again, we elect certain men whom we consider to be capable of assisting in the work of government for the benefit of all alike. Why is it that these supposedly wise persons still view the quack and the fake medicine man with non-seeing eyes in regard to the dangers of these robbers of the ignorant and credulous? We have with us, in quite a goodly number, the ministers of the Gospel, who teach that robbery is a crime in every respect. How does it come to pass that these moral teachers of ours either wink at the unscrupulous activities of the quack "doctor" or otherwise ignore the fact that such thievery exists to the moral and physical degradation of their congregations?

As the pharmacist is a co-partner with the physician in his work for the preservation of health and the cure and alleviation of disease, he, too, is acting toward the quack menace in just the same manner as those other educated and wise men mentioned above. The responsi-

*Reprinted from *Australasian Jour. of Pharm.*

bility for this condition of affairs rests as much with our profession as with any of the others.

We hear much of the desire of the politician to do all he possibly can for the general interest of the community, but when the thieving quack is mentioned these sentiments seem to be entirely "a horse of another colour." Still, these same men are well aware of the fact that obtaining money under false pretences is a punishable offence, and one that, committed by others than the quack "doctor," is punished severely.

Here we have a state of affairs where the quack is not only allowed to prosper at the expense of the public health and welfare, but is robbing that public outrageously in the bargain. In addition to this wholesale thievery, winked at by persons in authority, whose business it is to prevent anyone being made the victim of such dishonesty, the medical and pharmaceutical professions allow these fakirs to eat into their own callings to the extent of vast sums of money every day in the week, and yet they enter but feeble and haphazard protests.

Surely the Government officials, physicians, pharmacists, the press and the clergy of all denominations cannot be ignorant of the "dark and dangerous" ways of the unscrupulous quack or the bogus nostrum seller. Every one of these learned and educated persons knows that the quack menace is ever present in our communities, and yet little is done to run these fakirs out of business. Is it that the physician and pharmacist do not care either for the health or welfare of the public, and cannot understand that, with such dangerous persons out of the way, their professions would come into their own, by reason of their special training and experience?

When a quack does business with the credulous and ignorant it is at the expense of the doctor and pharmacist, whose rightful privilege it is to practise the healing art and to compound and dispense medicines. Any other person who obtains money from a sick person by false claims is a fakir, and does so illegally.

The legal machinery is in the hands of Government officials in all civilised countries, and there is no excuse for not making a bold attempt to bring these quacks to justice and get rid of them completely. The interests of the public demand that those in authority should protect the credulous and ignorant. This applies in force to the clergy. In regard to the medical profession and the profession of pharmacy, it is about time that these professions recognised their responsibilities in regard to the menacing quack. If wisdom, science, special training

and experience cannot function in the interest of the great majority and afford protection to the ignorant and credulous, then our much-vaunted civilisation is a cold-blooded and absolutely selfish one.

While those who know better allow the quack to exist among us, they practically condone his offence against the public health and welfare. Wisdom and learning have their responsibilities, and these rest upon the shoulders of those who are cognisant of the dangers of the present conditions. It is not enough for the decent-minded man to know; he is called upon to use his knowledge for the benefit of those who are incapable of discriminating. Observers state that quackery in Australia flourishes like the green bay tree and is practically unrestrained. This makes it all the more necessary to tackle the problem in a concerted way. To sit down and do nothing is to evade our responsibilities as citizens. Men and women are but "children of a larger growth," and many are as ignorant and credulous as they were in early youth. It is the duty of those who do possess superior knowledge to protect these childlike thinkers against the quack robber who preys upon them.

ORIGINAL ARTICLES

STUDIES ON LICORICE ROOT AND LICORICE EXTRACT

PART 4—A NEW SUBSTANCE IN CHINESE LICORICE ROOT

By Percy A. Houseman and Clement K. Swift

PREVIOUS PAPERS in THIS JOURNAL [1] by one of us have dealt principally with methods of analysis for different kinds of licorice root and extract, as well as with the distribution of constituents dissolved by organic solvents.

No detailed chemical investigation of these various constituents has yet been undertaken, with the exception of the work of Tschirch [2] and his pupils on the characteristic sweet principle—glycyrrhizin. Tschirch has claimed that glycyrrhizin is the di-glucuronic ether of glycyrrhetic acid, though it seems possible that he was dealing with galacturonic rather than glucuronic acid.

[1] 1912, **84**, 531; 1916, **88**, 97; 1921, **93**, 481.
[2] *Arch. d. Pharm.*, 1907, **245**, 97; 1908, **246**, 545; 1909, **247**, 121.

In the course of work on the resins and bitter principles of licorice root we have isolated from Chinese licorice root a new substance of the formula $C_{20}H_{21}O_9$. We have not found this substance in other varieties of the root, though it may be present in very small amount.

Preparation of New Substance $C_{20}H_{21}O_9$

Ten kilograms of shredded Chinese licorice root were placed in the continuous extraction apparatus of Houseman and Swift.[3] The root was dried for six hours by turning steam at six pounds per square inch pressure into the jacket and leaving the head off the percolator. After cooling, forty liters of 95 per cent. grain alcohol were added to the root in the percolator and the extraction was carried on for sixty hours. After recovering the alcohol, the brown semi-liquid residue was treated with ten liters of boiling water and the mass boiled down to about three liters to remove the remainder of the alcohol. The residue was then treated with four liters of boiling water. At 80 degrees C. the supernatent liquor was decanted from the soft resinous mass, which was then given a second and third treatment with four liters of boiling water each time.

The twelve liters so obtained deposited some further resin on cooling, and this resin was separated by decantation and also boiled three times with water, using 1500, 1000 and 500 cc. of water respectively.

From the total volume of fifteen liters of aqueous extract, there resulted on concentrating to about five liters, a total yield of twenty-seven grams of crude crystals, including second and third crops. (0.27 per cent. of the root used.)

Purification of the crude substance is readily achieved by crystallisation from boiling water, of which 200 times the weight of the substance is required for solution. Animal charcoal is of no assistance in the purification.

The substance also crystallises from absolute methyl and ethyl alcohols, but owing to its easy solubility in those solvents, the crystallisation is less convenient than from boiling water, as well as entailing greater loss. The substance crystallises from water in needles and melts at 202-204 degrees C. (uncorr.). It is optically active:—a_D (in absolute alcohol) $= +$ 48.8 degrees.

[3] *Ind. Eng. Chem.*, 1925, 17, 830.

Molecular weight

1. *Titration with* $\frac{N}{10}$ *KOH* (phenol-phthalein indicator), assuming 1 mol. potash to 1 mol. substance, gave molecular weights of 404 and 406. Calculated for $C_{20}H_{21}O_9 = 405$.

2. *Boiling Point Method*

	Mol. Wt.
(a) 0.3294 subst. in 23.8 g. abs. ethyl alcohol raised b. p. 0.034° C.	462
(b) 0.3559 subst. in 28.75 g. nitrobenzene raised b. p. 0.152° C.	407
(c) 0.4129 subst. in 28.50 g. nitrobenzene raised b. p. 0.186° C.	390
Calculated for $C_{20}H_{21}O_9$	405

ANALYSIS

Substance	0.1755	0.1743	0.2103	0.2396	
H_2O	0.0851	0.0843	0.1024	0.1145	
CO_2	0.3818	0.3791	0.4651	0.5175	
Found:					
Carbon	59.32	59.30	59.91	59.10	Average 59.41
Hydrogen	5.38	5.37	5.42	5.30	Average 5.37
Calculated for $C_{20}H_{21}O_9$					
Carbon			59.26		
Hydrogen			5.18		

REACTIONS

1. *Fuming nitric acid* yields picric acid.

2. *Iodine and Bromine* are readily absorbed and from the latter reaction a material was obtained which darkened at 136 degrees C. and melted at 137-138 degrees C. with evolution of gas.

3. *Potassium permanganate* readily oxidises the substance in alkaline solution, 0.1 gram requiring 18 cc. of normal $KMnO_4$. On adding alcohol, boiling, acidifying with HCL, filtering and applying the usual test with resorcinol, there developed a strong green-red fluorescence, indicating the formation of phthalic acid.

4. *Zeisel Reaction* was negative. The dark red residue from the Zeisel test was soluble in absolute alcohol, with which it was evaporated twice. On boiling with water a distinct odor of cinnamon was noticed. The red amorphous residue is soluble in ammonia, but forms no ammonium salt. This red phlobaphene-like substance, dissolved in alcohol, dyes silk and wool (both mordanted and unmordanted) brown to red shades.

The aqueous extract mentioned above deposits an orange amorphous solid on evaporation and cooling. The odor of cinnamon is again apparent, and when the mass has been evaporated almost to dryness there is sudden decomposition, with evolution of iodine.

5. *Distillation with Zinc Dust* was inconclusive. Both the liquid and solid portions of the distillate gave yellow-green fluorescence in ethereal solution. We believe a naphthalene ring to be present.
6. *Hydrogen peroxide* is without action.
7. *Ferric Chloride* gives a greenish yellow color, but no precipitate.
8. *Lead acetate and silver nitrate* do not form salts.
9. *Liebermann's reaction* for phenols was negative.
10. *Fehling's solution* is slowly reduced on long boiling.
11. *Millon's Reagent* gives a clear claret red, becoming turbid brown on boiling.
12. *Fusion with Caustic Soda* followed by extraction with ether gave negative tests for phloroglucinol, hydroquinone and pyrocatecol, but a strong positive test for resorcinol with sodium hypochlorite. The ether extract purified by crystallising from water gave fern-like white plates melting above 240 degrees C.
13. *Acetylation.*
    (a) 2 grams substance were boiled for forty-five minutes with 5 cc. acetic anhydride then boiled with water, evaporated to dryness, dissolved in alcohol and dried.
    Yield of acetyl derivative=2.901 grams=145%
    Calculated for 4 acetyl groups =141.5%
    On dissolving the acetyl derivative in a little glacial acetic acid, adding water until permanently turbid, warming, and allowing to stand, small needle-shaped crystals were deposited in ball-like clusters after two hours. Melting point of acetyl derivative 165-170 degrees C.
    (b) The method of Sudborough and Thomas (J. C. S. 1905, 1752) hydrolysing the acetyl derivative with benzene sulphonic acid and titrating with barium hydrate indicated 146.2% acetyl derivative. N
    (c) Titration of the acetyl derivative by hydrosis with 2 alcoholic potash gave 145% and 146% acetyl derivative.

The substance $C_{20}H_{21}O_9$ therefore contains 4 Hydroxyl groups. No combustion was made on the acetyl derivative because the

analytical figures of the acetyl derivative are practically the same as those of the parent substance $C_{20}H_{21}O_9$.

14. *Sodium hydrate* (cold 10% solution) dissolves the substance to a yellow solution, from which it is precipitated unchanged by carbon dioxide. The substance is insoluble in sodium bicarbonate. These tests indicate the absence of carboxyl groups, and that the acid character of the substance is phenolic.

15. *Hydrolysis.*

Hydrolysis with mineral acids or with caustic alkalis (aqueous or alcoholic) follows essentially the same course. A summary is given below.

(a) *Hydrolysis with 10% alcoholic potash.* When the substance is boiled with about ten times its weight of 10% alcoholic potash, a clear red solution is obtained with a little brown precipitate. After neutralising with hydrochloric acid the solution deposits yellow prisms melting at 185-189 degrees C.

(b) *Hydrolysis with 10% aqueous potash.* Three grams substance were treated one hour cold and one hour boiling with 100 c.c. 10% potassium hydrate. Neutralised with conc. hydrochloric acid, added a few drops of potassium hydrate, and made slightly acid with acetic acid. Shook out with ether.

(1) *Ether layer* gave 1.8 grams (60% yield). Phenolic odor. The aqueous solution of this material gave a first crop of yellow crystals melting at 177-180 degrees C., and a second crop from which on recrystallising two other kinds of yellow crystals were obtained, melting respectively at 124-127 degrees C. and 135-137 degrees C. The acetyl derivative of the crystals melting at 124-127 degrees C. is soluble in water.

(2) *Aqueous layer* on drying and extraction with a mixture of alcohol (3 parts) and ether (1 part) gave a yellowish syrup with a sweet and acid taste which gives a brown color with ferric chloride and reduces Fehling's Solution on boiling.

(c) *Hydrolysis with Mineral Acids.* The substance heated with ten times its weight of conc. hydrochloric acid gives a clear

red solution. At the boiling point a brick-red semi-liquid precipitate forms and chlorine is evolved. The brown residue is soluble in alcohol and ammonia, slightly soluble in ether. A similar material is obtained by boiling for four hours with 10% hydrochloric acid. An ammoniacal solution dyes silk a fine old-rose color and mordanted wool a yellowish brown. The hydrolysis product from dilute hydrochloric acid gave the following distribution on shaking out with ether.

(1) *Ether layer* gave orange crystals which melted at 190-192 degrees C., after crystallising twice from water. Yield 46% of original substance. The orange crystals are readily soluble in ether, alcohol, acetone. They crystallise well from chloroform, ethylacetate or acetone. They are readily soluble in ammonia to a deep yellow solution and are reprecipitated on boiling off the ammonia.

On heating the orange crystals with sodium hydrate solution, neutralising and shaking out with ether, a dirty blue color is obtained with a chip of wood and hydrochloric acid, and a dark green color with ferric chloride (indicates a substituted dihydric phenol).

(2) *Aqueous layer* on neutralising and evaporating gave a viscous sweet residue similar to that obtained by hydrolysis with potash. This syrup reduced Fehling's Solution and ammoniacal silver nitrate and gave a poorly-defined osazone.

Hydrolysis by boiling with dilute (1 to 3%) sulphuric acid follows a similar course. A yield of 57 per cent. of yellow crystals was obtained, readily purified from water, and melting at 190-191 degrees C. after three crystallisations.

The other hydrolysis product, obtained after removing the sulphuric acid with barium hydrate and barium carbonate, is a straw-colored sweet syrup, apparently the same as that resulting from hydrolysis with hydrochloric acid or with potash.

The Schotten-Baumann reaction on the yellow crystals gave a sticky white precipitate which deposited white crystals from chloroform solution. The amount was too small for further investigation.

The following table summarises combustions on recrystallised yellow crystals melting at 189-191 degrees C., obtained by acid hydrolysis of the original substance $C_{20}H_{21}O_9$. Molecular weight determinations using nitrobenzene are also given.

	1	2	3	Calculated for $C_{15}H_{14}O_4$
Acid used	10% HCl	1% H_2SO_4	1% H_2SO_4	
Carbon	69.60	69.52	69.46	69.77
Hydrogen	5.78	5.12	5.05	5.42
Molecular weight	238	223	250	258

Acetylation of these yellow crystals gave colorless needles, m. p. 175-177 degrees C., and the yield indicated two (OH) groups replaced by two ($COCH_3$) groups. Hydrolysis with magnesium hydrate converts the acetyl derivative to the original yellow crystals melting at 187-189 degrees C.

Alkaline potassium permanganate oxidised the yellow crystals to phthalic acid.

It was felt that the yellow crystals melting at 190 degrees C. and of probable formula $C_{15}H_{14}O_4$, resulting from the acid or alkaline hydrolysis of the original compound $C_{20}H_{21}O_9$, would furnish the key to the constitution of the latter.

A search of the literature indicated close relationship to *Lapachol* and its derivatives.

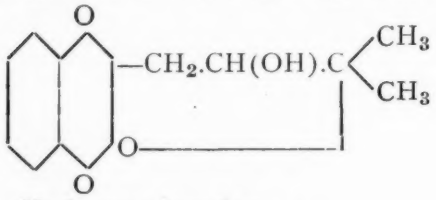
Hooker, in a series of papers,[1] discusses constitutional formulæ for lapachol, hydroxy-a-lapachone, hydroxy-b-lapachone, hydroxy-hydrolapachol, dihydroxy-lapachol and its anhydride, and hydroxy-isolapachol.

Our yellow crystals, melting respectively at 124-127 degrees C., 135-137 degrees C. and 189-191 degrees C. have very similar properties to Hooker's lapachol derivatives, but they did not quite check up with all of Hooker's conversions.

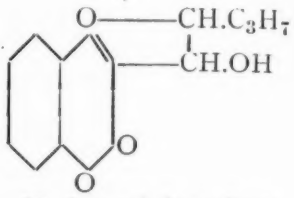
For example, hydroxy-a-lapachone, $C_{15}H_{14}O_4$, is described as yellow crystals, M. P. 187 degrees C.; acetyl derivative m. p. 179 degrees C. Our yellow crystals are $C_{15}H_{14}O_4$, melt at 189-191 degrees C.; acetyl derivative m. p. 175-177 degrees C. The parallel is close and the appearance of the crystals similar.

[1] *J. C. S.* 1892, **61**, 611; 1896, **69**, 1370-1381.

Hooker gives the following constitutional formulæ for hydroxy-a-lapachone and hydroxy-b-lapachone:



Hydroxy-a-lapachone

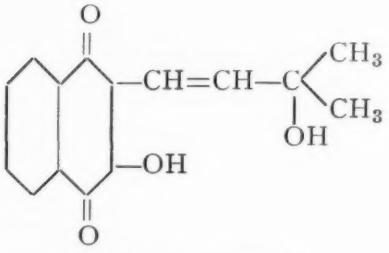


Hydroxy-b-lapachone

The parallel between our yellow crystals and hydroxy-a-lapachone was made closer by repeating Hooker's conversion of hydroxy-a-lapachone to dihydroxy-hydrolapachol. This last substance melts at 182 degrees C. and when our yellow crystals underwent a similar conversion, the resulting product melted at 183 degrees C.

For hydroxy-iso-lapachol, Hooker also gives the formula $C_{15}H_{14}O_4$, and considers it identical with Lomatiol, a yellow substance obtained by Rennie[2] from *lomatia ilicifolia* and *longifolia*. The melting-point of hydroxy-iso-lapachol is given as 134 degrees C, and our second crop of crystals from the ethereal layer of the potash hydrolysis melted at 135-137 degrees C.

The constitution of hydroxy-iso-lapachol is given by Hooker as:



Hydroxy-iso-lapachol

It seems probable therefore that our yellow crystals $C_{15}H_{14}O_4$, (derived from the hydrolysis of the substance $C_{20}H_{21}O_9$ found by us in Chinese licorice root), are closely related to hydroxy-a-lapachone.

Pressure of other work compels us to postpone completion of this investigation.

[2] *J. C. S.* 1895, **67**, 784.

Bertrand Schneeberg and Albert Spiegel have kindly assisted in the experimental work, and the MacAndrews and Forbes Company has generously supported it.

Camden, N. J., July, 1929.

ILLICIUM RELIGIOSUM, SIEBOLD*

Mang Tsao

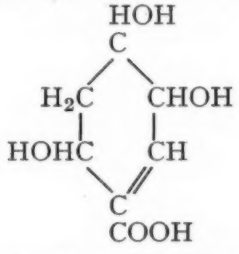
A PHYTOCHEMICAL STUDY

By Sze Yee Chen

(*Conclusion*)

Shikimic Acid

History. Shikimic acid was first isolated by Eykman [1] in 1885 from the fruit of *Illicium religiosum,* the poisonous star anise, the *shikimi* of the Japanese, hence the name. In the following year he reported its occurrence in the fruit of *Illicium verum,* the ordinary start anise.[2] In the former it is present to the remarkable extent of 18 p.c. In 1891 Oswald [3] reported on the acid in connection with his chemical study of true star anise, and in the same year Eykman [4] published a detailed study of its physical and chemical properties, assigning to it the structural formula



a cyclohexene (1) triol (3, 4, 6), carbonic acid (1).[5]

*Thesis submitted for the degree of Doctor of Philosophy, University of Wisconsin, 1927.

[1] *Trav. Chim. d. Pays-Bas* 4 (1885), p. 32-54.

[2] *Ibidem,* 5, p. 299-304.

[3] *Arch. d. Pharm.* 229 (1891), p. 84.

[4] *Ber.* 24 (1891), pp. 1278-1303.

[5] Meyer & Jacobson, *Lehrb. d. org. Chem.* II, 1, (1923), p. 855.

Physical properties. Shikimic acid crystallizes in micro-needles melting at 184°; $d_{14°} = 1.599$; $[\alpha]_D = -246.3°$; conductivity at infinite dilution = 303; heat of neutralization 13.1 cal. per mol. It is readily soluble in water (18 p.c.) less in alcohol (5 p.c.)—the supersaturated solutions do not crystallize—still less in absolute alcohol (2 p.c.) and diacetone alcohol and almost insoluble in absolute ether (0.015 p.c.) chloroform, benzene and heptane.

Chemical properties. This monobasic acid decomposes carbonates readily. Its potassium, calcium, magnesium, lead, strontium, and barium salts have been obtained in amorphous condition.

Its copper salt is soluble in concentrated sodium hydroxide and can be used for the testing of reducing sugars. The sodium and ammonium salts crystallize in beautiful rhombic sphenoids, while the methylamine, benzylamine, aniline and pyridine salts are obtained in the amorphous condition.

When its salts are heated shikimic acid may lose two molecules of water yielding p-hydroxy benzoic acid

$$C_6H_6(OH)_3COOH \xrightarrow{2H_2O} C_6H_4(OH)COOH$$

or two molecules of water and one molecule of CO_2 yielding phenol

$$C_6H_6(OH)_3COOH \longrightarrow C_6H_5OH + 2H_2O + CO_2$$

The presence of three hydroxy groups is indicated by the formation of triacyl (acetyl, propinyl, butyryl) esters.

The double bond takes on

(1) Hydrogen (Na amalgam and palladium H), yielding dihydroshikimic acid which should be isomeric with hexahydrogallic acid.

(2) Bromine, yielding dibromshikimic acid. This, in turn, yields a brom lactone by splitting off HBr, a reaction that would suggest a different structural formula than the one adopted by Eykman; also dioxy shikimic acid when treated with $Ba(OH)_2$. Dihydroxyshikimic acid is accordingly a quercite carboxylic acid.[6]

Preparation. The shikimic acid for this study was obtained from the fruit of *Illicium religiosum* by extracting with alcohol. The first part of the alcoholic extract, from which most of the solvent had been removed, solidified on cooling. After the removal of the chlorophyll and oils by means of percolation with acetone 827 grams of shikimic acid from 17.5 kilos of crude drug were left as white powder. From the remaining alcoholic extract 2,260 grams more acid were recovered

[6] *Ibidem*, p. 851.

by the same treatment. This portion of the acid has a brown color. The total yield of crude shikimic acid from the fruit is, therefore, about 17.5 per cent.

The crude shikimic acid can be purified by recrystallization from either water or alcohol. The supersaturated aqueous solution made by heating deposited on standing in an open vessel fairly pure acid in extremely fine particles. The complete deposition of the acid from the aqueous solution took about ten days. In case the solution was over concentrated by evaporation on a water bath, a solid cake was suddenly formed, or if the solution contained too many impurities, or was left for spontaneous evaporation, a syrup resulted.

As this acid is more soluble in water than in alcohol, an attempt to precipitate it from aqueous solution with alcohol was tried but proved a failure, no separation taking place at all. When one part of ether was added to two parts of saturated alcoholic solution of shikimic acid a fine precipitate formed; but if too much ether was used, or if the solution contained an appreciable amount of water and impurities, the acid separated together with the impurities in fine particles or as a liquid layer. The best way was found to crystallize it from 95 p.c. alcohol.

The crude shikimic acid was gradually added to hot alcohol with stirring until the formation of a saturated solution, to which charcoal was added. After refluxing for two hours on a hot plate the solution was filtered and left to stand over night. The crystalline mass was then freed from the mother liquid and dried. After one crystallization from alcohol the shikimic acid was found to have the following constants:

m.p.	183°	Eykman 184°
A.V.	313.8	
M.W.		" 174.0
Sp. Rotation of 7.27 p.c. solution at 25° =	175°	" 183.0°

Ammonium salt. Shikimic acid was neutralized with ammonium hydroxide, a slight excess of the base being used. The solution was set aside for spontaneous evaporation. It dried as a crystalline mass. This mass was dissolved in water to make a saturated solution and evaporated in a vacuum. When supersaturation had been reached, a few crystals of the salt were added resulting in solidification in a few minutes. This crystalline mass was again treated with a small amount of water not enough to dissolve all of the salt and allowed to

stand in a covered vessel. After two days fairly large rhombic crystals were obtained.

Sodium salt. This salt was prepared by neutralizing the crude shikimic acid with sodium hydroxide with phenolphthalein as indicator, and purified by means of charcoal. It has the same crystallizing property as the ammonium shikimate, that is it dries to a solid mass on evaporation in vacuum over sulphuric acid, or solidifies within a few minutes when its supersaturated aqueous solution is in contact with some of its crystals. It could not be obtained in definite crystalline form in this way. Indeed, Eykman claimed that the shikimic acid salts of the alkali metals had only been obtained in amorphous condition. It was found, however, that when alcohol was added to an aqueous solution of sodium shikimate in moderate concentration and the salt separated was redissolved by heating, beautiful needle-shaped crystals were formed on cooling. Crystals about one cm. long have been obtained in this way. The size of the crystals depends upon the concentration of the aqueous solution and the amount of alcohol added. The addition of alcohol to a saturated aqueous solution separated the salt in an aqueous layer beneath the alcoholic solution, with only very few small crystals separating from the upper alcoholic solution.

Water is the only solvent in which sodium shikimate is known to be soluble. This salt melts at 268° with decomposition. Treatment with 1:1 HCl and 1:1 H_2SO_4 did not yield shikimic acid.

Calcium shikimate. This salt was made from milk of lime and shikimic acid in aqueous solution. The slight excess of the base was removed by passing in carbon dioxide. Upon evaporation with a gentle heat the aqueous solution formed a thick sticky syrup which dried after long standing. The addition of alcohol to the aqueous solution always resulted in the separation of an aqueous layer.

Copper salt. The three hydroxy groups in shikimic acid suggest a similarity of this acid to tartaric acid which is used to hold cuperic hydroxide in Fehling's solution. For this purpose the following experiment was performed. Instead of an alkaline Rochelle salt solution, 50 cc. of an alkaline solution containing 9 grams of shikimic acid and 15 grams of potassium hydroxide was prepared. When this solution was added in equal volume to a copper solution, having the same concentration as in Fehling's solution, the precipitate first formed redissolved on shaking. When heated with glucose this mixed solution deposited metallic copper. Cuprous oxide was also

formed when this mixed copper solution was allowed to stand or upon heating.

Methylamine salt. Three cc. of 30 p.c. alcoholic methylamine solution and 6 grams of shikimic acid also in alcoholic solution were brought together and warmed on a water bath for half an hour under a reflux condenser. After the removal of most of the solvent by evaporation, a brownish precipitate was formed. It had a melting of 162°.

Dimethylamine salt. Ten cc. of a 20 p.c. alcoholic dimethylamine solution and 6 grams of shikimic acid, also in alcoholic solution were mixed and warmed for half an hour. On evaporation of the solvent, a reddish-brown syrup was left.

Pyridine salt. Shikimic acid dissolves readily with development of heat in pyridine. On evaporation of most of the solvent a crystalline powder was left. It melted at 184°. It had a brownish color and its aqueous solution reddened litmus paper.

Aniline salt. Shikimic acid is less soluble in aniline than in pyridine. When equivalent amounts of both were heated together on a water bath for two hours and then left to cool a brown substance was formed. Heating of the aniline with an alcoholic solution of shikimic acid produced the same substance. Both melted at 178°. It is soluble in water and the aqueous solution shows acid reaction to litmus paper.

Piperidine salt. Shikimic acid is insoluble in piperidine. When the mixture was heated, a yellow plaster-like material was formed. Heating of an alcoholic solution of shikimic acid with piperidine yielded a reddish brown syrup after the removal of the solvent and of the slight excess of piperidine by evaporation.

Benzoylation of shikimic acid. Five grams of shikimic acid were dissolved in 30 cc. of 15 p.c. sodium hydroxide solution and 16 cc. of benzoyl chloride were added. The mixture was shaken in ice to prevent a rise in temperature, the liquid at the top of the alkaline solution was separated, washed with water and crystallized from alcohol. A thick liquid was always the result. The alkaline solution gave an abundant amount of benzoic acid on acidification. Addition of sodium hydroxide solution to the mixture of benzoyl chloride and aqueous solution of shikimic acid, as directed by Houbon and Weyl, produced the same substance.

Since the resulting mass had an odor of the unchanged benzoyl chloride another experiment was made. Shikimic acid, an excess of benzoyl chloride and a great excess of sodium hydroxide solution

were mixed and shaken. When the flask became hot it was cooled by immersing in ice water. After the reaction had been completed, the excess of benzoyl chloride was destroyed by heating the mixture on a water bath until no more odor of this volatile substance was observed. On cooling a semisolid cake was formed at the top of the alkaline solution. After repeated recrystallization from hot methyl alcohol a white powder was obtained. It melted at 110–112°. The yield was very small. According to Eykman the yields of acetyl, proponyl and butyric esters of shikimic acid are very poor.

Tests. 1. Shikimic acid reduces cupric sulphate in strong alkaline solution to metallic copper; this reaction is accelerated by heat.

2. When heated with an ammoniacal silver nitrate solution, it produced no visible change.

3. When heated with an ammoniacal solution of mercuric acetate, a black precipitate is produced.

4. Shikimic acid decolorizes potassium permanganate solution very rapidly.

5. Shikimic acid absorbs bromine, but does not decolorize a solution of iodine in potassium iodide.

Bromination. To a cold 50 p.c. aqueous solution of shikimic acid made with the aid of heat, a calculated amount of bromine was added, the last few grams of the bromine colored the reaction mixture red. Crystals appeared at the bottom of the beaker after 24 hours and the amount reached its maximum in one week when kept in an ice box. After having been kept in the ice box for three months, no more crystals separated and they were, therefore, filtered off by suction. After recrystallization from alcohol the crystalline compound turned brown at 189° and melted at 214–215°. According to Eykman [1] the dibrom-shikimic acid turns brown and melts at 188° (uncorrected).

The mother liquid, of a syrupy consistency, retained its reddish color when kept at zero temperature and turned dark on exposure to room temperature. Treatment with alcohol yielded some precipitate which after recrystallization from a small amount of alcohol had the same crystalline form and melted at the same temperature as the dibrom-shikimic acid obtained above.

Addition of iodine. A few drops of iodine solution were added to an aqueous solution of shikimic acid. The iodine color of the solution did not fade after two months. Evidently the shikimic acid does not take on iodine although it absorbs bromine very readily.

[1] *Ber.* 24, 1 (1891), p. 1278.

Hydrogenation. Dihydroshikimic acid has been prepared by Eykman by reduction with sodium amalgam. The procedure was, however, tedious and the yield low. Catalytic reduction was, therefore, resorted to. After a preliminary experiment, water was found to be a better medium than alcohol for this purpose. Twenty grams of shikimic acid were dissolved in 120 cc. of water (making a saturated solution), heat being used to hasten the solution. The cooled solution was placed in a pressure bottle (a magnesium citrate bottle was used) which contained 0.4 g. of palladium black. The palladium black was prepared from palladium ammonium chloride.[2]

The reaction bottle which contained the solution and the palladium black was then evacuated and filled with hydrogen and then again evacuated and connected with an improvised apparatus. All the stoppers were fastened to the bottles by wires. Hydrogen was filled into the apparatus and pressure indicated by rubber ribbons on the manometer. After having found that the whole apparatus was air tight, the reaction bottle was set in motion in a motor shaker. When the manometer showed that no more hydrogen was being absorbed, usually after 3 to 5 days, depending upon the amount of catalyst used, the palladium black was filtered off and dried over H_2SO_4 in a desiccator for further use. The filtrate was evaporated in a vacuum desiccator. The concentrated solution yielded beautiful crystals which after recrystallization from water melted at 180° and its 10 p.c. aqueous solution in a 100 mm. tube turned the plane of the polarized sodium light to −1.08° at 26.5° or $[\alpha]_D = -12.8°$. Eykman obtained 175° (uncorrected) as the melting point and −18.2° as the specific rotation of dihydroshikimic acid.

Oxidation with potassium permanganate. To a 1 p.c. aqueous solution of shikimic acid a 1 p.c aqueous solution of potassium permanganate was gradually added with constant shaking until the pink color remained stable. The insoluble precipitate formed was filtered off. On spontaneous evaporation of the filtrate no crystalline compound separated, but a dark brown semi-solid mass resulted. This was readily soluble in water but not in any organic solvent. An attempt to effect purification by precipitating the aqueous solution with acetone was without success.

Oxidation with hydrogen peroxide in the presence of ferrous sulphate. According to Fenton, hydrogen peroxide in the presence of ferrous salt can oxidize hydroxy acids to aldehyde or ketone acids,

[2] Bayer, *Ann.*, 269 (1892), p. 189.

decarboxylation usually taking place at the same time. Glycollic acid, for example, is thus converted into glycoxalic acid; tartronic acid into meso-oxalic acid. The ketone acids thus formed can be condensed with phenylhydrazine acetate to form phenylhydrazones and thus identified. Since shikimic acid has three hydroxyl groups this reaction was tried.

Five grams of shikimic acid and 2.6 grams of ferrous sulphate were dissolved in water and to the aqueous solution 20 cc. of 3 p.c. hydrogen peroxide were gradually added. After the reaction had been completed, the solution was found to possess a reducing property toward Fehling's solution. Extraction with ether did not remove anything. A part of the solution was treated with a mixture of phenylhydrazine hydrochloride and sodium acetate and heated in boiling water for two hours but no crystalline hydrazone separatec out even on cooling.

The remaining solution was, therefore, evaporated to dryness and the residue extracted with alcohol, the insoluble inorganic salt filtered off and the solution evaporated to dryness again. Treatment with acetone resolved the dried residue into soluble and insoluble parts. After the removal of the solvent both parts were found acidic to litmus paper. The acetone-soluble part was readily soluble in alcohol, water and in hot ethyl acetate. It reduced Fehling's solution very readily. It was treated with warm ethyl acetate to move some insoluble matter. When the solvent was removed by evaporation some crystals were found in the oily residue. These crystals were dried over porous plate and their melting point was found at 175–178°. Heating with phenylhydrazine reagent in boiling water for one and a half hours did not give any crystalline compound.

Oxidation with ferric chloride. A few drops of ferric chloride solution were added to an aqueous solution of shikimic acid solution and the color of the inorganic salt did not disappear after three months, neither was a precipitate formed.

Preparation of phenacyl ester. To 5 cc. of an aqueous solution containing 5 g. of shikimic acid and 1.25 g. of sodium carbonate 5 cc. of an alcoholic solution containing 5 g. of bromoacetophenone were added and the mixture was heated on a water bath for one hour. No solid or crystalline substance separated out on cooling, neither after the addition of water. The alcohol was then distilled off and the residue extracted with choloroform which dissolved all of the yellow coloring. After recrystallization from alcohol the residue left upon evaporation of the chloroform was obtained as white crystalline com-

pound melting at 54°. Bromoacetophenone melts at 56°. Since no halogen could be detected in the reaction mixture by means of silver nitrate, it is evident that no reaction had taken place.

To repeat this experiment 1 cc. of a saturated aqueous solution of shikimic acid, previously neutralized with sodium carbonate was refluxed upon a water bath for two hours with 10 cc. of an alcoholic solution containing an equivalent amount of bromoacetophenone. The aqueous and the alcoholic layers did not mix. On cooling the aqueous layer (lower) solidified. The solid substance dissolved very easily in water, but not in any organic solvent. No halogen could be detected by means of silver nitrate. It decolorized potassium permanganate solution, and melted at 267° with decomposition. Sodium shikimate melts with decomposition one degree lower. It is again evident that no reaction had taken place in this experiment.

After sodium shikimate had been refluxed with bromoacetophenone in absolute alcohol for four hours, the unchanged salt was identified in the insoluble residue and the other reagent in the alcoholic solution.

Heating with α-pinene. Thirty cc. of α-pinene were heated with an excess of shikimic acid on a water bath for three hours. After filtering off the shikimic acid, the pinene was found to have a rotation in 100 mm. tube of −18.85°, while the pure pinene without the shikimic acid heated for an equal period had a rotation of −18.86°. Evidently little or no reaction had taken place.

Since the solubility of shikimic acid in pinene is very small (0.077 g. per 100 cc. of the saturated solution at 23°) prolonged heating was thought desirable.

An equivalent amount of shikimic acid and α-pinene were mixed in a pressure flask and heated on a water bath for one week. At the end of that period the pinene had acquired a yellowish color and the acid had become a semi-solid, sticky mass. The liquid part was filtered and its saponification value was found to be 6.71. The S. V. for the saturated solution of shikimic acid solution (0.077 g. per 100 cc.) is 0.27.

The S. V. shows that some reaction had been taking place but the amount was very small and no further investigation was made.

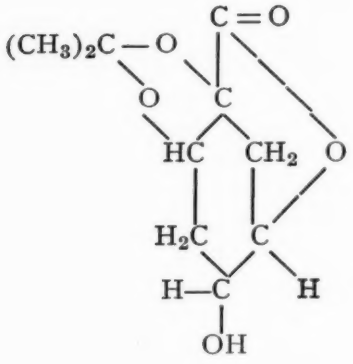
The residue was insoluble in all organic solvents except alcohol, but readily soluble in water. Both the alcoholic and aqueous solutions formed semi-solid masses but no crystals. Possibly it consisted of unchanged shikimic acid and some resinous reaction product.

Acetone shikimic acid. Since Fischer[1] first prepared acetone quinic acid the method of preparation has been simplified and the yield much improved (70 p.c).[2] As shikimic acid is closely related to quinic acid the corresponding compound was prepared in accordance with the directions of Fischer and Taube.[2] Fifteen g. of $ZnCl_2$ were dissolved in 100 cc. of commercial acetone and the impurities removed by filtration. To the solution 10 g. of shikimic acid were added and the mixture was shaken in a shaker for two hours. At the end of the period the shikimic acid went into solution. After two days beautiful crystals separated out from the solution melting at 186°. They are soluble in acetone, alcohol, ether, ethyl acetate and water but insoluble in chloroform. The aqueous, alcoholic and ethereal solutions were found to possess an acid reaction as indicated by phenolphthalein. The acid value of this compound was determined in aqueous solution:

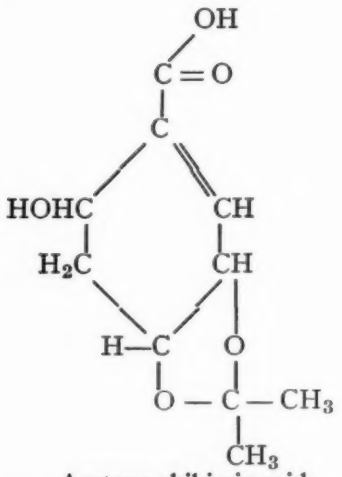
Sample	(1)	0.1989 g.	A. V.	260.6
	(2)	0.2298 g.	A. V.	257.8
		Average		259.2

The acid value of $C_7H_{10}O_5 + CH_3COCH_3 - H_2O$ is 259.6.

Assuming that the acetone shikimic acid is analogous to acetone quinic acid its composition may be expressed by the following structural formula:



Acetone quinic acid



Acetone shikimic acid

[1] H. O. L. Fischer, *Ber.*, 54 (1921), p. 775.

[2] H. O. L. Fischer and Taube, *Ber.*, 60 (1927), p. 487.

The strong acidity of the acetone shikimic acid in aqueous as well as in alcoholic and ethereal solutions seems to exclude the lactone formation, which, therefore differentiates it from acetone quinic acid. This may also be looked upon as further proof of the adopted structural formula of shikimic acid.

Physiological examination of shikimic acid. The nontoxicity of the shikimic acid and the readiness with which it is dehydrated to a hydroxy benzoic acid make it a matter of interest to ascertain the fate of this peculiar substance in the animal body. For this purpose a small amount of shikimic acid was taken orally and the urine was examined for its acidity and its hippuric acid content. The diet was controlled one day before the normal urine was collected and continued until all the acid urine had been obtained. Three hours after the administration of 0.3000 g. of shikimic acid another dose of the same amount was taken. A slight headache and a little loss of appetite were felt. Since both the normal and acid urine decolorized potassium permanganate solution and reduced Fehling's solution slightly, no evidence as to the presence or absence of the free shikimic acid in the urine was obtained. The results are herewith tabulatd:

Acidity of the urine:		*Normal urine*		*Acid urine*
Amount collected in 24 hours		1300 cc.		1160 cc.
Samples for acidity		25.00 cc.		25.00 cc.
1.0560 aq. KOH	(a)	6.1 cc.	(a)	8.55 cc.
10	(b)	6.1 cc.	(b)	8.70 cc.
Average		6.1 cc.		8.63 cc.

The total acidity in the normal urine is equivalent to 335 cc. N/10 KOH. The total acidity in the acid urine is equivalent to 423 cc. N/10 KOH. Increase in acidity equals 88 cc. N/10 KOH.

0.6 g. of shikimic acid is equivalent to 34.5 cc. of N/10 KOH. The high increase in acidity may be due to acidosis caused by this strong shikimic acid, and this acidosis may also account for the headache and the loss of appetite. Although the formation is still essentially unexplained, the conjugates of most aromatic alcohols, such as phenols, indoxyl, scatoxyl, pyrocatechol and the substituted

members of the group with sulphuric acid are not uncommon.[1] It is, probable, therefore, that the p-hydroxy-benzoic acid which may be formed from the shikimic acid through dehydration, conjugates with sulphuric acid and thus increases the acidity of the urine.

Hippuric acid. For the determination of this acid in urine Folin and Flanders's method,[2] as described in Mathew's Physiological Chemistry, 3rd edition, p. 1100, was used. For the sake of greater accuracy 300 cc. instead of 100 cc. were used. The total amount of hippuric acid in the 24 hour's urine were found as follows:

Normal urine	0.413 g.
Acid urine	0.940 g.
Difference	0.5271 g.

If all the shikimic acid be converted into benzoic or p-hydroxylbenzoic acid and all the newly formed acid into hippuric acid or its hydroxy derivative and all the hippuric acid excreted within 24 hours the increase of hippuric is calculated to be 0.6170 g.

Phytochemical study of shikimic acid. Since shikimic acid is present in the fruit of *Illicium religiosum* as high as 18 per cent., it was thought to be a kind of reserve material instead of a waste product. Cyclization of heptose accompanied by oxidation and dehydration may produce this acid. Armstrong[1] indeed, classifies shikimic acid together with quinic acid and inosite as a group of cyclic carbohydrates. Inosite has long been regarded as a cyclic sugar alcohol and quinic acid can be converted by *Aspergillus niger*[2] into a reducing sugar. It may, therefore, be assumed that shikimic acid is a carbohydrate-like derivative which is not highly toxic to the plant. Hence the toxicity of shikimic acid on plants was examined. For this purpose Macht and Krantz's method[3] for the phytopharmacological study of digitalis assay was used.

The seeds *Pisum sativum* v. *arvense* (Canadian field pea) of uniform size were sorted and germinated in quartz sand for three days when the length of the root had reached from 1 to 3 cm. After recording the exact length of the root, each seedling was placed in an

[1] Mathew, *Physiol. Chem.*, 3d ed., p.763.
[2] *Jour. Biol. Chem.* 11, 1912, p. 257.
[1] Armstrong, *The simple carbohydrates and glucosides*, 1924, p. 117.
[2] Kostyschew, *Zeitschr. f. physil. Chem.*, 111 (1920), p. 236.
[3] *Jour. A. Ph. A.*, 16 (1927), p. 210.

upright test tube containing a mixture of a nutrient solution and shikimic acid. The nutrient solution employed was a so-called Shive solution [4] which was made by dissolving 0.515 g. of $cáNO_3$, 1.80 g. of $MgSO_4$ and 2.45 g. of KH_2PO_4 in 1 liter of distilled water.

For comparison, the normal growth of the seedlings was determined by using equal parts of distilled water and the Shive solution. Then a 2 per cent. solution of shikimic acid was made up by mixing equal parts of a 4 per cent. aqueous solution of shikimic acid and Shive solution. A 1 per cent. solution of shikimic acid was made by taking equal amounts of the 2 per cent. solution and Shive solution. Other solutions of different concentrations are made from the more concentrated ones of shikimic acid and the Shive solution. The following solutions were made:

a. 2.0	p.c.	f. 0.05	p.c.	
b. 1.0	"	g. 0.02	"	
c. 0.5	"	h. 0.01	"	
d. 0.2	"	i. 0.005	"	
e. 0.1	"	j. 0.000	"	(pure Shive solution)

A series of 5 tubes was prepared for each concentration. After all the solutions had been made and all the seedlings had been measured and immersed in them, the plants were placed in a dark room at ordinary temperature. After a period of 24 hours the seedlings were examined and their length measured, and the loss of water in the tubes was made up by the addition of distilled water. The roots in the acid solutions did not grow in the first 48 hours but became thinner, possibly due to the high concentration of the solutions which caused the outward osmosis. Some plants died at that time. On the fourth day new root tips and leaves had been formed except in the first three series, *viz.* a, b, and c, in which all the roots had rotted and the cotyledons dried. No sign of growth was apparent. Left for three weeks, however, all grew fine with many leaves, long stems and numerous rootlets. Plants in the series e, f and h grew better than the others but the differences between the series were not very marked.

Comparison of the relative toxicity of shikimic acid and benzoic acid. In general the toxicity of acids toward plant life is directly proportional to the acidity or the conductivity of the acids. The last experiment has shown, however, that the shikimic acid, which

[4] *Physiol. Review*, 1, (1915), p. 327.

is a strong organic acid, has little inhibitive effect on the plant growth if the concentration is not too high. The toxicity of shikimic acid was, therefore, compared wth that of benzoic acid.

Again the seeds of *Pisum sativum* v. *arvense* of uniform size were germinated in quartz sand for three days and seedlings about 2 inches in length were selected. A set of five of them was placed in the five holes of a cork which was used as a stopper of a bottle in such a way that the seed of the plants rested on the cork and the roots reached the solution in the bottle. The bottles were amber-colored and 250 cc. in capacity. The solutions were prepared as follows:

Series I

	0.019 mol shikimic acid (3.31 g. per liter)	*Shive solution (diluted with an equal volume of water)*
A 1	100 parts	0 parts
A 2	80 "	20 "
A 3	60 "	40 "
A 4	40 "	60 "
A 5	20 "	80 "
A 6	0 "	100 "

Series II

	0.019 mol benzoic acid (2.32 g. per liter)	*Shive solution (diluted with an equal volume of water)*
B 1	100 parts	0 parts
B 2	80 "	20 "
B 3	60 "	40 "
B 4	40 "	60 "
B 5	20 "	80 "
B 6	0 "	100 " (Same as A 1)

Series III

C 1	0.1	mol shikimic acid			(1.74 p.c.)
C 2	0.02	"	"	"	(0.34 ")
C 3	0.005	"	"	"	(0.087 ")
C 4	0.001	"	"	"	(0.0017 ")
C 5	0.0002	"	"	"	(0.00034 ")
C 6	0.00005	"	"	"	(0.000087 ")
C 7	0.00000	"	"	"	(pure water)

Series IV

D 1	0.024	mol	benzoic	acid	(saturated	)
D 2	0.020	"	"	"	(0.24	p.c.)
D 3	0.005	"	"	"	(0.06	")
D 4	0.001	"	"	"	(0.012	")
D 5	0.0002	"	"	"	(0.0024	")
D 6	0.00005	"	"	"	(0.0006	")
D 7	same as C 7, pure water.					

After having been immersed in the solution in the bottles, the seedlings were placed in the dark room. After 24 hours all the roots immersed in the shikimic acid solutions were found thinner and surrounded with some fungus-like material, the amount of which was directly proportional to the concentrations of the acid in the solutions, and increased on standing. The roots in the benzoic acid solutions retained their normal shape but became darker. With the exception of B1, D1 and D2, all the roots and stems showed some growth but at different rates, best in pure water and worst in the most concentrated acid solutions. The stems developed better than the roots.

After 48 hours the roots in the shikimic acid solutions had accumulated more fungus-like material and those in the benzoic acid solutions had become darker. The length of the roots did not increase in A1, B2, C1, D1 and D2 and rootlets were formed in A3, A4, A5, A6, B4, B5, C5, C6, C7, D5 and D6.

After two weeks the original roots of A1 and A2 had completely rotted and were covered with something like a spider-net but with a few short new rootlets, while those in B1, B2, B3, D1, D2 and D3 were black and rotten without any rootlets or other material. The growth of the root of the rest of the plants were fine, D6, C7, C6, B6 or A6 being the best, and B1, B2, C1, D1, D2 and D3 dead. The stems grew better than the roots, and those in shikimic acid solutions were better than those in benzoic acid solutions. The accompany picture shows the growth of series I and II.

Repetition of the experiment on the comparison of the relative toxicity of shikimic acid and benzoic acid. Since in the last experiment the bottles and corks used were not sterilized, the fungus-like substance produced on the roots immersed in the shikimic acid solutions was thought to be due to infection by some organisms. Because of this infection it is difficult to tell whether the inhibitive effect upon the growth of the plants was due to the acid or to the organisms. The

experiment was, therefore, repeated using steam sterilized bottles and wax paper instead of cork for the covering of the bottles.

The seeds were germinated in the same way but longer. The seedlings used had root about 3 inches long and a stem about the same length. The concentrations of the solutions were slightly changed as stated below:

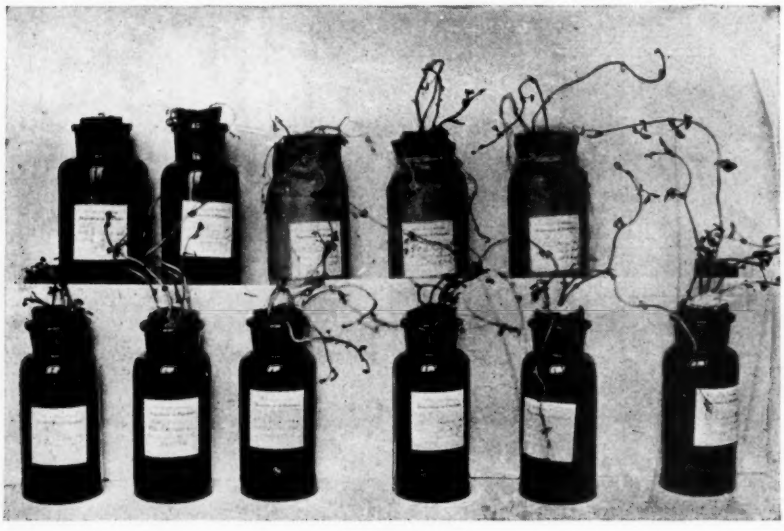


Fig. 4

Series I

	0.019 mol shikimic acid (3.31 g. per liter.)	*Shive solution (diluted with an equal volume of water)*
A 1	100 parts	0 parts
A 2	75 "	25 "
A 3	50 "	50 "
A 4	25 "	75 "
A 5	0 "	100 "

Series II

	0.019 mol benzoic acid 2.32 g. per liter.)	*Shive solution (diluted with an equal volume of water.)*
B 1	100 parts	0 parts
B 2	75 "	25 "
B 3	50 "	50 "
B 4	25 "	75 "
B 5	0 "	100 "(Same as A 5)

Series III

C 1	2.0	p.c. shikimic acid solution
C 2	0.2	" " " "
C 3	0.02	" " " "
C 4	0.002	" " " "
C 5	0.0002	" " " "
C 6	0.0000	" pure water

Series IV

D 1	0.27	p.c. benzoic acid solution (saturated)
D 2	0.2	" " " "
D 3	0.02	" " " "
D 4	0.002	" " " "
D 5	0.0002	" " " "
D 6	same as C 6	

After the roots had been immersed in the solutions for 24 hours a little fungus-like substance was found on the roots in the shikimic acid solutions but none in the benzoic acid solutions nor in pure Shive solution. Four plants died in A1, five in B2, B3, D1, and D2; three in B3 and C1; and two in B4. The difference was most striking in C2 and D2 and a photograph of them was, therefore, taken to visualize it.

After one week the difference in the rate of growth was even more marked as is again visualized by a photograph.

Fig. 5

Fig. 6

Conclusion. Although the absolute toxicity of shikimic acid has not yet been definitely determined, the fact that this acid has a much less inhibitive effect upon plant growth than benzoic acid is certain. What is most interesting to note is that shikimic acid, which is more favorable to the plant growth, has a greater acidity than benzoic acid.

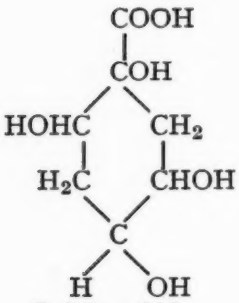
That the infection of the root occurred only in the shikimic solutions and not at all in the benzoic acid solutions shows that shikimic acid is more favorable to the growth of lower organisms than benzoic acid. It may, therefore, be suggested that, different from the ordinary strong acids, shikimic acid is closely related to carbohydrates, to which it is closely related, and into which it is possibly converted and then utilized by plant organisms.

The action of molds upon shikimic acid

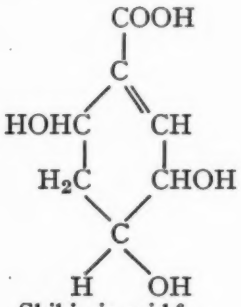
Kostyschew [1] in 1904 showed that, when cultured in a nutrient solution, *Aspergillus niger* can utilize quinic acid as source of carbon and that a reducing sugar is formed in the culture medium. Later,[2] in 1920, the same author proved the reducing sugar to be glucose by means of the osazone. Among the fermentation products alcohol was also found. In 1924 Butkwitsch [3] showed that besides *Aspergillus niger, Citromyces glaber, Aspergillus oryza* and *Mucor racemosus* can grow on salt solutions containing quinic acid. In the early stage of growth 3, 4-dihydroxybenzoic and 1, 2-dihydroxybenzoic acid were found and isolated. According to the same author quinone and hydroquinone were also probably present at this stage, but later these substances disappeared and oxalic acid appeared simultaneously in increasing amount. The oxalic acid in turn, was oxidized to carbon dioxide. The fungi which were able to utilize quinic acid are among those which transform citric acid into sugar. In the following year [4] he found that those fungi which can utilize quinic acid as source of carbon assimilation can oxidize glucose into gluconic acid. These experimental facts have led him to suggest a similarity between the metabolism of quinic acid and that of carbohydrates.

As shikimic acid and quinic acid have similar structures as shown by the following formulae:

[1] *Jahresb. f. Wiss. Botanik* 40 (1904), p. 575.
[2] *Zeits. f. physiol. Chem.*, 111 (1920), p. 206.
[3] *Biochem. Zeits.*, 145 (1924), p. 442; *Chem. Abstr.* 19, p. 91.
[4] *Ibidem*, 149 (1925), p. 385-413; *Chem. Abstr.* 20, p. 1929.



Quinic acid [5]



Shikimic acid [6]

The utilization of shikimic acid by molds was similarly studied to find whether the molds can, by decarboxylation and cleavage of the ring or some other means, convert this acid into a reducing sugar and to find what are the intermediate benzoic acid derivatives in the reaction mixture. The reducing sugar may throw some light upon the similarity between the shikimic acid and carbohydrates and the benzoic acid derivatives may check the structural formula of the shikimic acid which has not yet been definitely proved.

*Fermentation by Clostridium thermocellum.** This organism has fermentative powers on a large number of carbohydrates, including pentose, starch and cellulose, in a different degree but does not attack malic and citric acids to any extent.[7] It was chosen for the study of the similarity between the metabolism of shikimic acid and carbohydrates.

The nutrient medium (Levy Well's) was prepared according to the following formula:

Monobasic potassium phosphate	1 g.
Sodium ammonium phosphate + 4 H_2O	2 g.
Magnesium sulphate, crystals	0.3 g.
Sodium chloride	0.1 g.
Peptone	5 g.
Ferric chloride	traces
Distilled water	1000 cc.

[5] *Jahrsb. f. Chem.*, 1925, p. 168; Karrer, *Helvetica Chim. Acta*, 8 (1925), p. 202; *Chem. Abstr.* 19, p. 1269.

[6] Eykman, *Ber.* 24 (1891), p. 1278.

* This experiment was carried out following the directions of professors W. H. Peterson and E. B. Fred and the culture work was done by Mr. E. A. Marten. To them the writer is much indebted.

[7] Peterson, Fred and Marten, *J. Biolog. Chem.* 52 (1926), p. 309.

The acid was dissolved in the salt and peptone solution to make a concentration of about 1 per cent. and then sterilized. This medium was inoculated with 1 per cent. of a 24 hour culture of the organism. Sterilized calcium carbonate was then added to maintain a suitable hydrogen ion concentration and the tube tightly capped with tin-foil and incubated at 65° C. for one week. A total of 20 cc. of the solution was used The culture medium was analysed for unfermented shikimic acid, volatile acid, alcohol and reducing substance. Due to the small amount of material available only qualitative tests were made.

Volatile acid and alcohol. The culture was filtered to remove the excess of calcium carbonate and a part of the filtrate was acidified with H_2SO_4 saturated with sodium chloride and distilled over a small flame. In the distillate no acidity was indicated by methyl orange. The alcohol was tested for by Nicloux's method.[8] To 3 cc. of the distillate three drops of a 1/10 N. potassium dichromate were added and shaken well. Concentrated sulphuric acid was then dropped into the mixture until the solution boiled. The color of the mixture was compared with a similar mixture made from 3 cc. of distilled water. No difference in intensity could be observed. While another tube containing the same amount of potassium dichromate and sulphuric acid and 3 cc. of 1:1000 diluted alcohol decolorized within a few seconds. According to Nichoux, this method can be used for the quantitative estimation of alcohol in a solution less than 1/1000.

Reducing substance. The residue after distillation was treated with Fehling's solution and heated. No precipitate was formed. The same result was obtained with the undistilled culture.

Unfermented shikimic acid. The filtered culture was found to decolorize $KMnO_4$ solution rapidly. The free acid and the calcium salt of shikimic acid give the same reaction. Five cc. of the filtrate were then evaporated on a water bath to dryness. The residue amounted to 0.0580 g. (The amount of calcium shikimate in 5 cc. of 1 p.c. solution is 0.0555 g., while the amount of calcium carbonate[9] in 5 cc. of water at 20° is 0.00006 g. or is negligible.) Ignited in a crucible it yielded 0.0075 g. of ash. The computed 0.0580 g. of calcium shikimate is 0.0081 g. It shows, therefore, that no fermentation had taken place.

[8] Bull. de la Soc. Chim. (3), 35 (1906), p. 330.

[9] Hoplemab, *Zeitschr. f. physik. Chem.*, 12 (1893), p. 129, 241; Seidell, *Solubilities of inorg. & org. compounds*, 1907, p. 86.

Comparison of the rate of fermentation of shikimic acid by various molds.

In this series of experiments it was planned to compare the fermentability of the shikimic acid by different molds so that the best one could be repeated on a larger scale for the study of the end-products.

Enough shikimic acid was dissolved in a nutrient solution made up according to the following formula so as to yield a 1 p.c. solution of the acid.

M.	Ammonium nitrate	250 cc.
M.	Monobasic potassium phosphate	100 cc.
M.	Magnesium sulphate	40 cc.
M.	Ferric chloride	2 cc.
M	Distilled water	608 cc.

Twenty-nine cc. of the nutrient solution were sterilized for half an hour and then inoculated with the mold at 28° C. After three weeks it was found that *Aspergillus niger* grew best and *Aspergillus* 598 next, both with good spores. They flourished on the acid solution as on ordinary carbohydrates. *Aspergillus fumigodis* grew fairly, while *Aspergillus flores* and Silage mold had but very little growth.

After the removal of the mold pad the cultures were filtered, saturated with sodium chloride and distilled. The distillates were tested for acidity by means of methyl orange, and for alcohol by means of potassium dichromate in the same manner as described in the case of *Clostridium thermocellum*. Neither alcohol nor acid were found in any of these cultures. After 24 hours the dichromate color in the distillate from cultures of *Asp.* 598, *Asp.* niger, and *Asp.* flores disappeared.

The residue after distillation was titrated with N/10 KOH using phenolphthalein as indicator. The results are here recorded:

Molds	*State of growth*	*cc. of culture taken*	*cc. of N/10 KOH used for titration*	*cc. of N/10 KOH for equal vol. of 1 p.c. acid sol.*	*p.c. of acid fermented*
Silage mold	poor	21.0	10.8	12.18	10.9
Asp. fumigodis	fair	19.0	5.28	11.02	52.9
Asp. 598	excellent	18.9	3.38	10.96	68.9
Asp. niger	excellent	21.0	3.54	12.18	70.8
Asp. flores	poor	18.5	2.12	10.73	80.2

With the exception of the last one the above results show that the least amount of acid was left in the culture where the mold grew the best.

The neutralized solutions were tested for reducing sugars by means of Fehling's solution but none showed any reduction.

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Pen Tsao Kang Mu (A natural history), 1596, chapter 17.

The author gives a general description of *Mang Tsao* particularly of the leaves which were the part used. It is said to be a fish poison and a rat destroyer. It is employed as anthelmintic and as anodyne. Its chief use is for dermatitis. The decoction should not enter the eye.

Kaempfer, Engelbertus. 1712.

Amoetitatum Exoticarum, p. 880.

The author fully describes the plant: fruit, flower and leaves of *somo* or *shikimi* and supplies a very good illustration. The use of *shikimi* in Japan is also recorded.

Bartram, John. 1769.

Shrubs and Plants; a description of East-Florida with a Journal kept by John Bartram of Philadelphia, 3rd ed. p. 16.

According to the author the star anise tree in Florida is identical with *somo* or *shikimi* of Japan. Later it was identified as *Illicium floridum.*

* The writer wishes to acknowledge his indebtedness to Professor W. O. Richtmann for his assistance in securing the references.

Linné, Carl. 1772.

Materia Medica, 1772, p. 140; Species Plantarum, 1797, p. 1254.

The author assumes that the star anise in the market and *Illicium anisatum* of Loureiro's *Flora cochinchinensis* are the fruit of *Shikimi* or *Somo* of Kaempfer's *Amoenitates.*

Loureiro, Joannes de. 1790.

Flora Cochinchinesis (1790), I p. 353.

The author describes the fruit, leaf, and flower of *Illicium anisatum* as the same as those of *Shikimi.*

Linné, Carl. 1825.

Systema Vegetabilium II, (1825), p. 643.

The statement is made that *Illicium* is indigenous to Japan and China.

Ainslie, Whitelaw. 1826.

Materia Indica II, (London), p. 18.

The author states that *Shikimi* or *Somo* of Japan and *Pa-Co-Hui-Hiam* (= Chinese for star anise) of China are synonyms of *Illicium anisatum.*

Hayne. 1856.

Getreue Darstellung und Beschreibung der Arzneigewaechse, Berlin, Bd. 12, p. 29.

A description of the plant and fruit of *Illicium anisatum* with a colored illustration. Although no difference between the true and poisonous star anise is given, the description and the picture indicate it to be the former.

Siebold, Philipp Franz. 1871.

Flora Japonica, p. 5.

A fairly detailed description, with illustration, of *Illicium Japonicum:* fruit, leaves, flower, the entire plant and its history.

Berg, Otto. 1878.

Pharmakognosie des Pflanzen-und Thiereichs, 5th ed. Berlin, p. 361.

According to the author the fruit of *Illicium religiosum,* v. Siebold, resembles that of the genuine star anise but is smaller. The shape and taste of the carpel are also recorded.

Fluekiger, F. A., and D. Hanbury. 1879

Pharmacographia, p. 20.

As an adulterant of star anise the *shikimi* fruit is mentioned.

Bentley, Robert. 1880.

Medical Plants, I, p. 10.

The author considers *Illicium religiosum* and star anise, *shikimi,* as synonyms of *Illicium anisatum.* An illustrated description is given.

1880.

Jahrb. ueber d. Fortschr. d. Pharmacie, 15, p. 50.

A general description of the whole plant, leaves, fruit and carpel is given.

Eykman, J. F. 1881.

Pharm. Jour. 40 (1880-1881), pp. 1046 and 1066.

Illicium religiosum, Sieb.; its poisonous constituents and essential and fatty oils.

The constants of the volatile oil, distilled from the leaves, are recorded. The author finds that the toxic principle is chiefly in the seed. The isolated shikimine is claimed to be the poisonous constituent. In the second paper the author describes the whole plants, the leaves and fruit of both *Illicium religiosum* and *Illicium verum*. The morphological differences between the seeds of the two species are specially mentioned.

Editorial, (Oberdoerffer, A.) 1881.

Pharm. Centrabl. 22 (1881), pp. 162, 177, 276, and 400.

Japanischer Sternanis, Giftiger Sternanis.

A description of the morphological differences between the true and poisonous star anise.

Vogl, Aug. 1881.

Mitth. des Wien. Med. Dokt. Colleg., 7, pp. 167-73.

Ueber giftigen Sternanis.

In this article the actual size, color, surface structure and the general shape of the fruit, carpel and fruit stem are fully described. The author also reports a histological study of the fruit.

Luerssen, Christen. 1882.

Handb. d. system. Botanik II, p. 583.

The author simply records the height of the tree and the color of the bark.

Eykman, J. F. 1884.

Recueil d. Travaux chim., 3, p. 204 [Ber. R. 17, p. 441].

Sur les principes actifs de la skimmia Japonica.

From the wood the author obtains skimmine, $C_{15}H_{16}O_8$, which is slightly soluble in water forming a neutral solution. Heating with a mineral acid converts the skimmine into skimmetine, $C_9H_6O_3$. Both skimmine and skimmentine are soluble in alkaline media and do not reduce Fehling's solution.

1884.

Jour. de Pharm. et de Chim. 118, p. 367.

Sur une Badiane dangereuse.

Two poisoning cases due to star anise which was identified as *Illicium religiosum*, are recorded.

Menier, Ch. 1884.

Jour. de Med. de l'Ouest. Nantes, 2nd s. 8, pp. 113-14.

Note sur une Falsification de l'Anis étoilé.

According to the author, Japanese and Chinese star anise, when mixed, can be easily separated following the direction of Holmes, odor and taste being the best guide. He also states that *Illicium religiosum* is frequently cultivated in the gardens of eastern France.

Moeller, Josef 1886.

Mikroskopie der Nahrungs-u. Genussm. aus dem Pflanzereiche, p. 276.

The author fully describes the structure of star anise and mentions the difference from Japanese star anise.

Tambon, J. 1886.

Des Illicium en Généra, de la Badiane et de son Huile essentielle en particulier, Montpellier.

In this thesis seven species of *Illicium: majus, parviflorum, griffithii, floridanum, religiosum, sanki,* and *anisatum* are fully described and illustrated. The author finds that chloroform yields more extractive from both carpel and seed of poisonous and genuine star anise than carbon disulphide. The carpel of *I. anisatum* yields 10 times as much of extractive as the carpel of I. *religiosum.*

Barral, E. 1890.

Jour. de Pharm. et de Chim. 21, p. 319.

Sur une Badiane toxique, Illicium Parviflorum.

The author states that the fruit of this plant, found in Anam, is highly poisonous.

Inoko, Y. 1890.

Chiugai Iji Shipo, (Medical News of the World), Tokyo, pp. 1245-1248 and pp. 1317-20.

Shikimi no Stsu (=The tree of Shikimi).

Synonyms, habitat and general description of the plant are recorded, also numerous poisonous cases. The symptoms observed in the poisoned people and in experimental animals are said to be similar to those of strychnine poisoning.

Pabst, G. 1890.

Koehler's Medizinal Pflanzen, 1, p. 117.

A colored illustration of *Illicium anisatum. Illicium religiosum* is given as as a synonym.

Fluekiger, F. A. 1891.

Pharmakognosie des Pflanzenreiches, 3rd ed. pp. 932-37.

Brief accounts of *Illicium religiosum* and *I. verum* are given.

Pfister, Rudolf. 1892.
Vierteljahrs. Naturforschr. Gesellsch., Zuerich, 37, pp. 313-22.
Zur Kenntniss d. echten und d. giftigen Sternanis.

Having examined different samples of *Illicium religiosum* the author concludes that the aleurone grains of the seed of this drug contain generally a large crystalloid and one or two globoids, while in star anise the aleurone grains contain globoids, rarely crystalloids.

Bartram, J. and Michaux. 1893.
Flora Bor. Am. 1, p. 32.

In this article *Illicium parviflorum* was mistaken for *Illicium religiosum.*

Schimmel and Co. 1893.
Berichte, Oct. 1893, p. 46.

The physical constants of the volatile oil from *Illicium religiosum* are recorded.

Waage, Th. 1893.
Ber. d. Pharm. Gesellsch. 3, p. 161.
Fructus Anisi stellati.

According to the author poisonous and true star anise when mixed can easily be distinguished by their morphological differences, but not by the odor since that of the one is contaminated with that of the other. The aleurone grain in the seed is said not to be a distinct feature.

Collin and Planchon. 1896.
Les Drogues simples d' Origine végétale. 2, pp. 891-92.

The authors supplement a description of Japanese star anise with a morphological as well as a histological illustration. Its difference from true star anise is also mentioned.

Tschirch and Oesterle.
Anatomischer Atlas d. Pharmakognosie u. Nahrungsm., pp. 241-44.

Under the title *Fruct. Anisi Stellati* the authors compare *Illicium religiosum* with star anise. Both morphological and histological descriptions are supplemented with very good illustrations. Special attention is given to the aleurone grains of the seed. A chemical test for the differentiation of the two species is also described.

Villiers and Collin. 1900.
Traité des Altérations et Falsifications des Substances Alimentaires, pp. 295-303.

Following the description of star anise, the authors give a detailed account of the morphology and histology of the Japanese star anise with illustrations.

1901.

New York Med. Jour., 73, p. 642.
Poisoning with star anise.

The case of a woman who had taken an infusion of star anise.

Beuttner, E. 1907.
Schweiz, Wochenschr. Chem. u. Pharm. 45, p. 277.
Giftiger Sternanis.

Following the directions given in Tschirch and Oesterle's *Anatomischer Atlas,* the author examined and fully described a mixture of true and poisonous star anise.

Schimmel and Co. 1909.
Bericht S. & Co., April, 1909, p. 51.
Oel von Illicium religiosum.

When cooled in ice mixture the oil solidified. Cineol, linalool and safrol were identified.

Kraemer, Henry. 1910.
Botany and Pharmacognosy, 4th ed. p. 275.

The differences between the stone cells of *Illicium religiosum* and *Illicium verum* are mentioned.

Bulir, Jaromir. 1912.
Zeitschr. f. Untersuchung d. Nahrungs-u. Genussm. 24, p. 309.
Zusammensetzung d. fetten Oele d. Samen von Illicium verum Hook. u. Illicium religiosum, Sieb.

The seed of *Illicium verum* yielded 13 p.c. and that from *I. religiosum,* 8 p.c. The entire fruit of *I. verum* yielded 2.6 p.c.; that of *I. religiosum* yielded 1.0 p.c. Oleic, linolic, stearic and palmitic acids were found and their percentages were computed.

Guirrero, L. E., D. de Pag., and A. L. Guirrero, 1916.
Philippine Jour. Sc. 11 B, p. 203.
Poisoning by Illicium religiosum Sieb.

Four cases of poisoning from the use of a decoction of *Sanki,* the fruit of *Illicium religiosum* are reported. According to an unpublished experiment carried on in the Department of Pharmacology, University of Philippines, the fruit of *I. religiosum* is fourteen times more toxic than that of *I. anisatum.*

Wall, Otto A. 1917.
Handbook of Pharmacognosy, 1917, p. 427.

Both *Illicium verum* and *I. religiosum* are illustrated. According to the personal experience of the author shikimi fruit can not be very poisonous, because no serious effects seem to have followed after having chewed freely of

a spurious star anise which consisted of at least one-third of its weight of shikimi.

Negrete, J. V. and C. F. Velarde 1919.

Semana Méd. Buenos Aires, 26, pt. 2, pp. 554-65.

Contribucion al Estudio de la Toxidad del Illicium religiosum (Anis estrllado del Japon).

From experimentation on different animals: dogs, rabbits, guinea pigs, pigeons, frogs, white rats and mistoes, the author concludes that young animals are more susceptible to the drug than old ones. Poisoning occurs in the central nerve system and the action is not peripheral. The poisonous symptoms consist of circular movement of the head, periodical violent convulsions, paralysis and death. They are comparable to picrotoxine poisoning. From equal amounts of the drug the half-an-hour cold infusion and half-an-hour decoction are equally toxic while 24 hour maceration at room temperature yielded a less potent preparation. The poisonous principle can be taken up by animal charcoal.

Read, B. E. 1922.

The China Med. Jour. July, 1922, p. 1.

Pharmacognostic Notes 1.

After having mentioned a few cases of poisoning from false star anise, the author describes morphological differences observed between this drug and true star anise.

Murakoshi. 1925.

Flora of Japan, p. 592.

The plant of *Illicium anisatum* of Japan is briefly described with illustrations. Its uses in Japan are also mentioned.

Chen, K. K. 1926.

Jour. A. Ph. A. 15, p. 861.

A study on Illicium religiosum.

The author records the physical properties of the volatile oil from the carpel, of the fatty oil from the seed, also moisture and ash determinations. The toxic principle is found to be partially destroyed by alkalies but not by acids. An attempt to crystallize it failed.

Chou, T. Q. 1927.

Chinese Jour. Physiol. 1, p. 213.

Shikimitoxin, the toxic principle of Illicium religiosum.

The author isolated shikimitoxin from the fruit of *Illicium religiosum* as a white amorphous powder sintering at 63° and completely liquified at 130°. 0.2 mg. per kilo are found to be the M. L. D. for rat.

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A QUALITY STANDARD FOR CROCUS

By E. H. Wirth*

STANDARDS FOR CRUDE vegetable drugs as set forth in the monographs of the Pharmacopœia and National Formulary may be classified into three divisions. First, those of identity evidenced in the definition, the macroscopical and microscopical descriptions and the various identity tests. Second, those of purity into which category fall the macroscopical and microscopical descriptions of adulterants, the chemical tests for adulterants and the ash, moisture and foreign organic matter limits. In short any process determining the presence of foreign substance is a purity test. Third, the determination of the quality of the drug. The use of the word "quality" in this case refers to the content of natural constituents of the drug upon which its action is dependent. Examples of this type of standard are the alkaloidal assays, the glucosidal assays, the various solvent extractives, etc.

Crocus with its picturesque history of adulteration and sophistication has apparently brought about the inclusion into its monograph

*Assistant Professor of Pharmacognosy, University of Illinois.

of many tests for the detection of adulterants, yet under the present-day administration of the Food and Drugs law it is doubtful whether a considerable quantity of adulterated saffron can be found on the market, even though the unscrupulous intent toward the adulteration of this drug due to its high price no doubt still exists. It is questionable, however, whether crocus of inferior quality is not frequently sold. It seemed, therefore, of interest to investigate the quality of crocus.

The tinctorial power or coloring action of crocus is due to a glucoside crocin which upon hydrolysis yields crocetin.[1] The therapeutic action is possibly also due to crocin, although some free crocetin as well as a small amount of volatile oil exist in the drug. Assuming that the value of crocus is directly proportional to its crocin content a colorimetric assay might be useful in the determination of its quality. Crocin being a glucoside might also be determined by the sugar formed upon its hydrolysis and methods [2] to this end have been suggested. Colorimetric methods are generally, however, considerably more simple to perform. In this connection Vinassa [3] suggested the use of potassium dichromate solutions as standards. Dowzard [4] estimated the crude crocin content of saffron colorimetrically using a standard chromic acid solution. He compared his solutions in Nessler tubes of equal bore. The first official inclusion of a colorimetric test seems to be that of the Pharmacopœia Nederlandica of 1905. Here it is stated that 10 mg. of saffron in 1000 cc. of water should be equivalent to 50 mg. of $K_2Cr_2O_7$ in 1000 cc. of solution. The latest edition of the German Pharmacopœia (Deutsches Arzneibuch 6. 1926) has adopted 0.05 gm. of $K_2Cr_2O_7$ in 100 cc. of water as a colorimetric standard for a 1-10,000 dilution of saffron. No other recent pharmacopœia available to the author gave colorimetric standards although many give statements similar to that of the present National Formulary 5 that a dilution of 1-100,000 gives a distinct color.

The author has studied a colorimetric method patterned after the official German one but has further employed the colorimeter, (B. & L. Biological No. 2400) in making determinations. Samples

[1] *Archiv. der Pharm.*, 252 (1914), 139.
[2] Pfyl and Scheits. *Unters. d. Nahrungs- u. Genusm.*, 16 (1908), 347.
[3] *Arch. Pharm.*, 1892, 353.
[4] *Pharm. Jour.*, 61 (1898), 443.

of various origin were chosen and used in the following colorimetric assay.

"Crocus is powdered (approx. #40) and dried in a dessicator. Weigh 0.100 gm. and place in a 100 cc. volumetric flask. Fill to the mark with distilled water and allow to stand three hours at room temperature with frequent shaking. Filter at the end of three hours and dilute one cc. of this filtrate to 10 cc. with distilled water. Compare this solution with a N/100 solution of potassium dichromate in a colorimeter." Standard.—A 1-10,000 solution of crocus prepared as above should show a depth of color not less than that of an equal depth in millimeters of a N/100 solution of $K_2Cr_2O_7$.

Six samples have been selected from those studied. They are as follows:

Sample A. A stock sample about three years old (U. of Ill.) kept in a closed tin container.

Sample B. Purchased in a retail store. Had been in stock about two years. Kept in a closed tin container.

Sample C. Purchased from a retail store. In stock less than a year. Kept in a closed tin container.

Sample M. Museum sample about eight years old. Kept in a stoppered glass container in dull light.

Sample L. A museum and lecture sample several years old, (possibly 20 years). Kept in a stoppered glass container but exposed to rather strong light.

Sample P. Sample about 15-20 years old. Kept in a closed tin container.

In the following table the first column gives direct readings in mm. of the 1-10,000 dilutions as compared with a 20 mm. depth of N/100 dichromate solution. The second column gives the factor using the N/100 $K_2Cr_2O_7$ as 1.00. This factor represents color strength above or below the selected standard.

Sample	*Direct reading*	*Factor*
A	15.1	1.324
B	15.2	1.316
C	13.1	1.526
M	42.8	0.467
L	125.0	0.16
P	30.0	0.66

Comments

The samples for which the above results are given were pure samples and the style content was within the National Formulary limit of 10 per cent. It was observed in all samples studied that previous drying in the dessicator (24 hours) did not make material difference in results when checked against similar but undried samples, and it is naturally advised that such drying is hardly necessary. If excessive moisture were present it would, of course, somewhat lower the final colorimetric result. It is very interesting to note that the tints of the crocus solutions and of the dichromate solutions are very nearly similar, at least so close that the colorimetric end point may be readily determined with ease. The method seems admirably adaptable and the use of the colorimeter gives very accurate results, at least much more so than could be obtained with tubes and the naked eye.

Whether the standard which the author has chosen (N/100 potassium dichromate solution) (0.4903 gm. per L.) is sufficiently high as an official standard is, however, open to question, and can only be determined by the examination of samples from various localities. Of the samples purchased in local stores none fell below this standard and one (Sample C) ran as high as 1.5. The average was about 1.3 or 30 per cent. over the standard. It will, however, be noted that the standard is approximately the same as the standards of the Netherlands Pharmacopœia of 1905 and the German Pharmacopœia of 1926. It should be further observed that in this type of work a certain amount of flexibility should be allowed and therefore minimum standards are not customarily placed high.

The three samples of considerable age (M, L and P) gave a wide range of results all considerably below standard. It will be noted, however, that those which have been exposed to light show much lower results. It can then be assumed that light is perhaps even a greater factor than age in the deterioration of crocus.

The Effect of Light on Crocus

The author has begun a series of experiments to determine the relative effect of light and age upon crocus which will probably involve a considerable period of time. Several samples of crocus have been prepared in closed containers some being exposed to light and some kept in darkness. These will be tested periodically and within

a few years will yield fairly accurate data upon this subject. Crocin in solution, however, shows a very rapid fading upon exposure to light. The filtrates (1-1000) from the assay process were divided into equal portions, one portion being exposed to light (in a north window) and the other being kept in the dark. At twenty-four-hour intervals one cc. was taken from each of these and diluted to 10 cc. with water. This dilution was compared in the colorimeter with a N/100 $K_2Cr_2O_7$ solution. All data is given on the basis of 1-10,000 solutions of crocus for 20 mm. of N/100 $K_2Cr_2O_7$. The following data are taken from the results obtained with samples A, B and M.

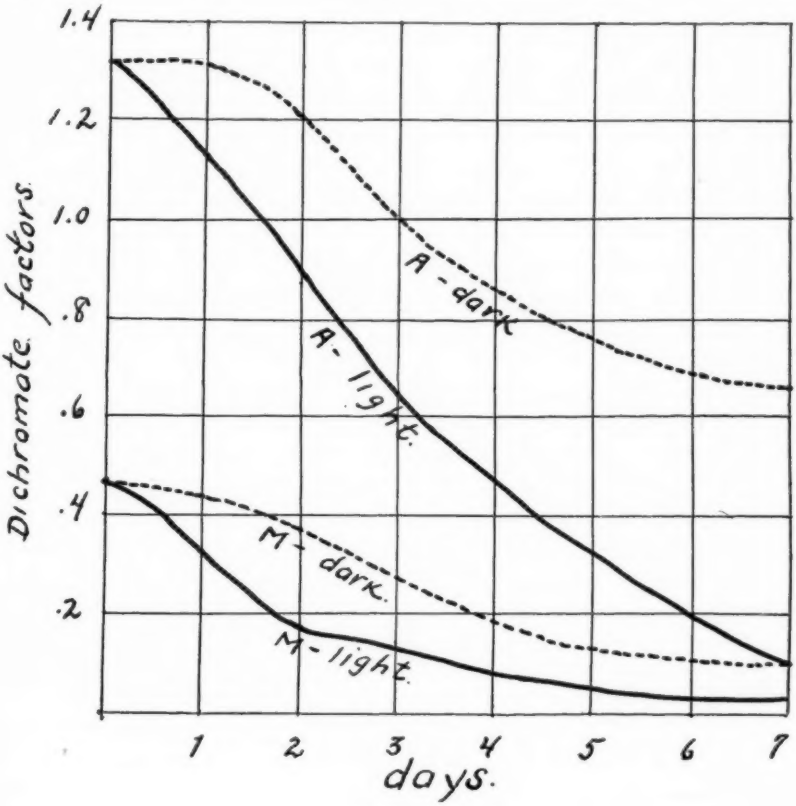
Reading in mm. Exposed to Light.				Reading in mm. Kept in the Dark.		
A	B	M		A	B	M
15.1	15.2	42.8	Immediately on dilution	15.1	15.2	42.8
17.8	18.0	60.0	After 24 hours (1 day)	15.2	15.3	46.4
23.0	22.6	108	After 48 hours (2 days)	16.6	16.5	54.0
31.0	28.0	140	After 72 hours (3 days)	20.0	19.5	60.0
200	165	800	After seven days	30.0	30.0	196

Factors. Light.				Factors. Dark.		
A	B	M		A	B	M
1.32	1.31	0.46	Immediately on dilution	1.32	1.31	0.46
1.12	1.11	0.33	After 24 hours (1 day)	1.31	1.30	0.43
0.87	0.88	0.18	After 48 hours (2 days)	1.20	1.21	0.37
0.64	0.71	0.14	After 72 hours (3 days)	1.00	1.02	0.33
0.10	0.12	0.025	After seven days	0.66	0.66	0.10

The curves show the effect of light upon solutions of crocin. The drop in tinctorial power for the first forty-eight hours is much greater in the solutions exposed to light. The curve for Sample B is almost similar to that for Sample A so is not given on the chart. The curves for A denote the behavior of an average normal crocus of good quality. The M curves indicate the behavior of a crocus of poor quality. While the drop in the latter is not so great due to the fact that a great deal of the tinctorial power has previously been lost in the crude drug, there is, however, a rapid drop especially during the first forty-eight hours. This loss of color is apparently due to the hydrolysis of the crocin as fading can be brought about very rapidly by boiling with dilute mineral acids. Whether the loss in tinctorial value (decomposition of the crocin) would give a similar

curve but at a much slower rate in the crude drug, will be determined by the experiments now under way. For the present it is fairly safe to assume that the results will be approximately the same.

The work, nevertheless, shows that there is a very distinct decomposition and that light as well as age is a distinct factor. Experiments are also under way to determine the effect of moisture upon the deterioration of crocus.



Relation of Styles to Stigmas

The fifth edition of the National Formulary allows a maximum of 10 per cent. of yellow styles. These styles also contain crocin but in much smaller amounts than the stigmas. Styles and stigmas were separated in several samples of crocus (mostly of the Alicante variety) which met the above described colorimetric standard. These were then powdered and submitted to colorimetric assay. In general

it was found that the stigmas contain from 17 to 19 times as much crocin as the styles. Average results are given below.

N/100 dichromate factors for stigma	1.4 to 1.6
N/100 dichromate factors for style	0.07 to 0.08
Crocin in stigmas as compared with styles	17.4-18.9 times.

Solvent Tests for Crocus

The National Formulary gives several solvent tests for crocus. The prime purpose of these tests is the detection of the addition of foreign coloring matter and has nothing to do with the quality determination. These tests might also, in a sense, be interpreted as identity tests, yet they are, as was stated, primarily purity tests. Solutions of 0.2 per cent. concentration (0.01 gm. in 5 cc.) in methyl alcohol, ethyl alcohol, acetone, chloroform and ether are employed in these tests and the corresponding colors of the solutions are described in terms of deep orange, lemon yellow, light lemon yellow and a slight yellow tinge. Solutions in carbon disulphide, carbon tetrachloride, xylene and benzine are said to remain colorless.

During the progress of the crocus study it was thought that the colorimetric study of crocus solutions in these solvents, while having no bearing upon the quality of the drug, might nevertheless be of interest. Solutions were prepared by placing 0.1 gm. of powdered crocus in a 50 cc. volumetric flask and filling to the mark with the solvent. These solutions were allowed to stand with frequent shaking for three hours, filtered and then submitted to colorimetric assay. It must be remembered that while we have employed solutions twenty times as concentrated as in the aqueous assay process, these solvents only extract a small amount of color. Results given herewith have been calculated for a 20 mm. depth of N/1000 $K_2Cr_2O_7$.

0.2% solution in	Depth in millimeters.	Factor (N/1000 dichromate)
Ethyl alcohol	0.12	16.3
Methyl alcohol	0.16	12.5
Chloroform	16.00	1.25
Ether	22.00	.90
Xylene	40	.5
Benzol	40	.5
Carbon tetrachloride	50	.4

The factors indicate the relation of colors imparted to the various solvents. The depth of color in the carbon tetrachloride solution, for example, is only about one-fortieth that of the alcoholic solution. (The color depth of the alcoholic solution in turn is only about one-sixteenth that of an aqueous solution.) Among the interesting facts which this solvent study brings out are:

(1) Solutions of crocus in xylene, benzol and carbon tetrachloride, while nearly colorless to the naked eye are readily perceptible with the colorimeter.

(2) The chloroformic solution shows somewhat greater depth of color than the etherial solution. The N. F. states the ether solution to be "very slight lemon yellow" and gives a "very slight yellow tinge" for the chloroformic solution, implying in its sequence that the chloroformic solution is of lesser color depth.

(3) The N. F. further states that the methyl-alcoholic solution is deep orange. In every case of methyl-alcoholic solutions prepared by the author they were yellow in color. There is, of course, considerable chance for error here as methyl-alcohol containing an appreciable amount of water might extract enough color to give a color similar to the aqueous solution which is orange in color. In all cases the color is yellow and it is questionable whether the term "lemon-yellow" is advisable. In greater concentration, as in the aqueous solution the color is orange but this upon dilution becomes yellow. The colors are, in fact, very similar to those of dichromate solutions.

Conclusions

The quality of crocus is discussed together with the effect of light and age upon the drug. A colorimetric method for the estimation of the quality of crocus is described and discussed. This method in short consists of macerating 0.1 gm. of powdered crocus in 100 cc. of water at room temperature, with frequent shaking for three hours, filtering, diluting ten times with water and comparing this dilution (1 in 10,000 of crocus) with an aqueous solution of potassium dichromate of definite concentration in a colorimeter.

A one-hundredth normal solution of potassium dichromate is suggested as a standard, although a slightly higher one might be shown to be preferable by subsequent investigation.

It is recommended that this method be incorporated into the crocus monograph together with a suitable standard.

In connection with the colorimetric assay the colors imparted by crocus to other solvents have been investigated and it is advised that this part of the crocus monograph also receive careful revision.

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REINECKE'S SALT AS A MICROCHEMICAL TEST FOR ALKALOIDS*

By L. Rosenthaler

Communication from the Pharmaceutical Institute of the University of Berne

THE IDENTIFICATION of alkaloids by conversion into microcrystalline compounds is well suited to replace tests by the well-known color reactions, although as yet the latter cannot be entirely dispensed with. The crystal precipitations are mostly as sensitive as the color reactions and more reliable being less influenced by impurities. However, a whole series of alkaloids may give with the same reagent similar precipitates, so that it is desirable to have more reagents. The greater the number of characteristic precipitates, that can be obtained with a given alkaloid, the greater the surety and reliability of the test.

Many precipitating reagents for alkaloids are found among acids and salts, the anion of which contains a heavy metal in complex combination. It was therefore to be expected, that characteristic alkaloid precipitations will also be obtained with Reinecke's salt. Christensen has obtained crystalline compounds with organic bases. The salt can be prepared by fusion from ammonium thiocyanate and potassium and ammonium bichromate and can easily be separated from this fusion. The cold saturated aqueous solution was used as reagent. It is not very stable and cannot be used if ferric chloride indicates the presence of CNS.

The reaction is carried out (with exception of piperin), thus: a little bit of the alkaloid or the alkaloid salt is placed on the glass

*Reinecke's Salt is a Complex Thiocyanate Derivative. O. F. Christensen (*J. Prakt. Chem.*, 1892, **45**, 213) assigns to it the formula:

$$Cr_2NH_3(CNS)_3 + NH_4CNS.$$

slide of the miscroscope with a drop of the reagent. In the following text, all cases that gave notably remarkable results are stated.

Arecoline hydrobromide. Aggregation of fine small needles.

Atropine sulphate different shaped aggregates with smooth or with uneven margin.

Berberine sulphate. Very slow formation of small needles, also in aggregates and small rosettes.

Quinine hydrochloride. Slow formation of rosettes from small and large needles.

Coniine hydrobromide. The main forms are rosettes, with a few uneven scattered crystals; some rhombic shaped crystals and plates. Very often large plates and needles were obtained (impurity?).

Hydrastinine hydrochloride. Rapidly formed aggregates and branched out needles.

Morphine hydrochloride. Immediate formation of rosettes and aggregates of needles.

Narceine. Immediate formation of aggregates of branched needles.

Pilocarpine (hydrochloride) formation of bush like arrangement of needles.

Piperin (dissolved in hydrochloric acid). Slow formation of aggregates of partly curved needles.

Strychnine hydrochloride. Gradually forming aggregates of simple and branched needles.

Stovaine. Rapid formation of needles and feather-shaped crystals.

Tutocaine. Gradual formation of aggregates and rosettes of small needles.

Novocaine hydrochloride. Gradual formation of aggregates and rosettes of leaf-like single crystals.

Alypin hydrochloride. Needles, partly single and partly in aggregates.

Cocaine hydrochloride. Very slow formation of rosettes and aggregates of very small needles. Occurs only on longer waiting and even then not always regularly.

Tropacocaine hydrochloride. Rapid formation of small rosettes and aggregates of needles.

MEDICAL AND PHARMACEUTICAL NOTES

CHEMICAL BIBLIOGRAPHIES—The National Research Council announces the publication of its Bulletin No. 71, which is the First Supplement to the Bibliography of Bibliographies on Chemistry and Chemical Technology covering principally the period 1924-1928, Bulletin No. 50 having covered the period 1900-1924. This Supplement contains approximately 4100 bibliographies classified under 1050 headings. The original Bulletin (No. 50) contained approximately 10,000 bibliographies classified under 2400 headings.

As the title indicates, the work (as in the case of Bulletin No. 50) is a compilation of bibliographies published as separates, or at the end of books or magazine articles, or as footnotes to the same, on the numerous aspects of pure and applied chemistry. Each entry gives name of author or compiler, title, and place of publication. The majority of the entries state the number of references, thus giving an indication of the completeness of the particular bibliography. The entries are classified under the proper subject-headings, alphabetically arranged. The duplication of individual entries has been largely avoided by the liberal use of cross references.

As an example of the value of this compilation, the following information is given regarding the number of bibliographies reported in Bulletin 71 for some of the more important topics:

> Blood chemistry 25, Cement 21, Coal 31, Colloids 55, Dielectrics 24, Dyes and Dyeing 27, Fertilizers 38, Fibers 26, Foods 33, Iron and Steel 76, Metabolism 77, Milk 43, Mineral Resources 25, Paper 39, Petroleum 54, Photography 36, Plant Chemistry 41, Rubber 55, Vitamins 25, Water 45.

Copies of Bulletin No. 71 may be obtained from the publication office, National Research Council, B and Twenty-first Streets, N. W., Washington, D. C., for $1.50 a copy. Also copies of the previous Bulletin (No. 50) may be obtained from the same source for $2.50 each.

KRATOM EATING—In the report of the Director of the Government Laboratory of Siam the following interesting note appears: Kratom leaves from *Mitragyne speciosa* are much used for chewing in Peninsular Siam, and, to a small extent, in Bangkok. Investigations of the physiological action of the alkaloid, mitragynine, are still in progress. According to the reports of the officials of the Revenue Department, the chewing of kratom leaves is habit-forming. Addicts appear to be able to endure great fatigue and exposure to heat. The habit has not a bad reputation, like opium smoking, nor does there seem to be any progressive change in the condition of the addict or in his character. On the other hand, educated people avoid the habit. Habitual eaters are thin, having unhealthy complexions and dark lips. No immunity from malaria is conferred. A fresh leaf weighs on the average 1.7 gm. and 0.43 gm. when dry, and contains about 0.2 per cent of mitragynine; other alkaloids appear to be present. When an excess of the leaves is eaten, vomiting and dizziness are produced; numbness of the body, twitching of the hands and feet, and an effect on the heart have also been reported. From ten to thirty leaves are usually taken from three to ten times a day, and water is drunk after chewing.—(*Pharm. Jour.*)

AUTUMN LEAVES RED BECAUSE OF AN ALCOHOL—Leaves turn red in autumn from the same cause that turns noses red at any time—alcohol. Such is the declaration of Samuel G. Hibben, lighting specialist of the Westinghouse Lamp Company, who has been investigating the effect of sunlight on living things. Frost, usually credited as the agent in autumnal leaf-color changes, really has very little to do with the case, Mr. Hibben says. Leaves age, and when they grow old they do not function as efficiently as they did when they were young. Certain chemical products accumulate, among them various alcohols, and these act on the other substances in the leaves to give the bright-colored pigments.—(*Science Service.*)

ADRENALIN ACTION EXPEDITED BY MASSAGE—Discovery by Dr. A. B. Luckhardt of the University of Chicago and Dr. Theodore Koppanyi of Cornell University Medical College here, that the powerful drug adrenalin forms depots when injected under the skin has opened the way for a new method of treatment of certain diseases.

Adrenalin has long been used to raise the blood pressure, particularly in cases of shock following severe injuries or operations, and because of its relaxing effect on the bronchial muscles it has been used effectively in treating bronchial asthma. However, to produce the desired effect, the adrenalin had to be injected directly into a vein, and for each attack a fresh injection of adrenalin had to be made.

Drs. Luckhardt and Koppanyi have shown in dogs that adrenalin is capable of elevating the blood pressure even if injected beneath the skin, but they have also discovered the conditions under which the blood pressure elevating effect of the adrenalin injected beneath the skin may be elicited. They found that about fifteen minutes after the injection of adrenalin underneath the skin, when the injected area was gently massaged, there was at once a very considerable and protracted rise in blood pressure.

Deep anesthesia militates against the effective elicitation of this response, and this is the reason why previous investigators failed to get blood pressure rises following adrenalin injections beneath the skin. Drs. Luckhardt and Koppanyi have pointed out that adrenalin injected beneath the skin remains there for some time, and it was even possible to produce blood pressure rises from massaging such areas which had been injected twenty-four hours before the massage.

Green Color in Plants Related to Blood—Another step in the solution of two fundamental problems of plant and animal life has been taken by Prof. Kurt Noack of Bavaria, who has just shown that chlorophyll, the green coloring matter of plants, is related to blood and is derived from a substance known to biochemists as protochlorophyll.

Chlorophyll is the most abundantly produced complex organic chemical compound on the earth upon which we live, said Dr. Frank M. Schertz of the United States Bureau of Chemistry and Soils here in explaining Professor Noack's discoveries.

The breaking down of the chlorophyll molecule releases energy that may be manifested in many different ways. Chlorophyll in some way as yet unknown to us is directly related to the dry weight of plants, to the mineral elements found in the soil and in the plants, to the organic compounds found everywhere about us, to the electromagnetic waves which come to our planet, and most mysteriously of all to every manifestation of life we find here on earth.

"A complete understanding of chlorophyll in all its relations will aid us greatly in understanding whence we come, and whither we go," he said. "Chlorophyll is so little understood, yet it is the most important compound in all the world. It seems to be derived from protochlorophyll, a peculiar red-fluorescing pigment which is produced in seedlings grown absolutely in the dark. Protochlorophyll changes photochemically into chlorophyll upon exposure to light. Chlorophyll is an oxidation product of protochlorophyll. It is with the origin of this vitally important substance, chlorophyll, that Professor Noack's experiments are concerned."

The work of Professor Noack has shown very clearly a relation between the green pigment found in leaves and the red pigment of blood, although just how the blood pigment is formed from the chlorophyll has not yet been determined.

How hemoglobin, the substance which gives the red color to blood, is formed from chlorophyll has been a much-disputed subject among scientists. Professor Noack's experiments lend definite support to this theory. In the galls of animals that have been fed on green fodder is found phylloerythrin, a substance basically related to hemoglobin. On the other hand, this phylloerythrin, which is formed in the animal body from the chlorophyll of the green fodder, is chemically very similar to protophæophytin in a direct derivative of protochlorophyll, the same pigment which constitutes the first step in the formation of chlorophyll itself.

The origin of chlorophyll, on which Professor Noack's work touches, is the first of two problems very important in biochemistry, Dr. Schertz commented. When scientists find how protochlorophyll is produced, this first problem will be solved. The second problem concerns the way in which blood pigment is produced from chlorophyll. These two important problems must sooner or later be solved by biochemists, as they are most fundamental in all plant and animal life.—(*Science Service.*)

Survey Shows Plenty of Potash—The survey now being conducted by the United States Bureau of Mines and the U. S. Geological Survey has disclosed the fact that we have in the Permian basin a sufficient reserve of potash to make the nation independent in any future emergency such as that which arose in 1910 when German

legislation voided all American potash contracts. It will be necessary to solve certain problems of transportation, marketing and mining engineering before the potash is available for commercial use, but these problems are now being attacked by Federal. state and private agencies.

The area in which the potash-bearing salts have been found occupies about twenty-two counties in Texas and New Mexico. Altogether it covers about 40,000 square miles.—(*Science Service.*)

MOONLIGHT SHADOW CAUGHT BY CHEMISTRY—Demonstrating the much-mooted effect of moonlight on starch by means of a chemical trap to catch a shadow was the feat reported before the meeting of the British Association for the Advancement of Science by Miss E. Semmens of Bedford College, London. Miss Semmens has for some years been studying the effect of polarized light on starch solutions, which it is claimed are turned into sugar by prolonged exposure. Moonlight is composed largely of polarized sunlight, and it might be expected that it would have an effect on suitably prepared starch.

Miss Semmens dipped slips of paper into starch solutions and exposed them to moonlight, covering parts of them with small opaque objects to cast shadows. Then she dipped the paper into an iodine solution, which as is well known turns starch black but leaves sugar colorless. Where the shadow had fallen, there was a faint but distinguishable deepening of the color, indicating that sugar had been formed in the uncovered part. She was also able to repeat the same test with leaves containing starch grains.—(*Science Service.*)

NEWS ITEMS AND PERSONAL NOTES

FELLOWSHIP IN PHARMACEUTICAL RESEARCH OFFERED IN PHILADELPHIA COLLEGE OF PHARMACY AND SCIENCE—A new fellowship in pharmaceutical research has just been established at the Philadelphia College of Pharmacy and Science. The fellowship carries with it a stipend of $1000 and free tuition. Applications are being received now from prospective candidates for the college year 1930-31. However, if an especially well-qualified person is avail-

able, it is possible that the holder may begin work at the start of the second term of this year, in February, 1930.

Persons holding the degree of Bachelor of Science in Pharmacy, Chemistry or related subjects are eligible for consideration. The fellowship not only offers the opportunity for original research under competent direction, but also the opportunity to pursue studies leading to the degrees of Master of Science (M. S.), Master of Pharmacy (Ph. M.), and Doctor of Pharmacy (Phar. D.). The term of appointment for the fellowship ordinarily will be for one college year, but a holder who shows unusual ability may be reappointed for one or more succeeding years, thus permitting him to obtain these higher degrees while holding the fellowship.

The new $500,000 building of the Philadelphia College of Pharmacy and Science contains the latest and most complete equipment for Pharmaceutical research; therefore the fellowship holder has at his command every facility for original and productive research. In addition, he will be in constant contact with leaders in research, both among manufacturers and others active in pharmacy.

The college invites application from qualified persons. Further information about the nature of the fellowship may be obtained by writing to the Philadelphia College of Pharmacy and Science at its new building, Forty-third Street, Woodland and Kingsessing Avenues, in Philadelphia.

AMERICAN PHARMACEUTICAL ASSOCIATION COMMITTEE ON RESEARCH—At the meeting of the American Pharmaceutical Association held at Rapid City, S. D., August 24-31, 1929, that organization's Committee on Research, after a study of seven requests for grants from the association's research fund, recommended the following awards:

1. To H. V. Arny, of the College of Pharmacy of Columbia University, for completion of the $2000 fund required to finance the two-year fellowship planned for the study of the deterioration of chemicals and pharmaceuticals under the influence of light. $200.00
2. To E. H. Kraus and F. F. Blicke, of the College of Pharmacy of the University of Michigan, for continuation of research on the phenol halo-phthaleins..... 200.00

3. To E. Kremers and S. Chechik, of the College of Pharmacy of the University of Wisconsin, for research on thymol derivatives..... 250.00
4. To W. J. Husa, of the College of Pharmacy of the University of Washington, for continuation of work on the stabilization of pharmaceuticals..... 100.00

These committee recommendations met the approval of the association at its general session.

The $750 available for research grants this year represent one-half of the annual interest on the A. Ph. A. research fund, a fund accruing out of the profits on the sale of the National Formulary, one of the publications of the association.

NEW ASSISTANT DIRECTOR OF MULFORD LABORATORIES—A recent announcement has been made of the appointment of Dr. Jose Zozaya as assistant director of the Mulford Biological Laboratories.

Dr. Zozaya, a native of Monterey, Mexico, was educated in the United States, at St. Mary's College, San Antonio, Texas, and later at St. Louis University. His study of medicine and interneship in the Jewish Hospital in St. Louis was followed by research work at Iowa University and at Washington University in St. Louis, and later by graduate work at Harvard University, where he obtained the degree of Doctor of Public Health in 1925.

Under a fellowship from the Rockefeller Foundation Dr. Zozaya then studied the organization and work of public health laboratories and agencies.

He was then called by the Mexican Government to organize the Institute of Hygiene in Mexico City. He became the director and is credited with the planning and building of the laboratories of the new Institute of Hygiene of Mexico and with the organization of its activities.

PARKE, DAVIS & CO. PURCHASE COLTON CAPSULE DIVISION—The president of Parke, Davis & Co., Mr. Oscar W. Smith, announces the fact that his company has just purchased the capsule division of the Arthur Colton Co., of Detroit. This latter concern has for a number of years been manufacturing empty gelatin capsules which are used in pharmacy as containers for medicinal agents. Parke, Davis & Co. have bought all of the Colton apparatus, including all of their

stocks and patents on capsule-making machinery. The Colton capsule plant will be removed bodily to the Parke, Davis & Co. laboratories in Detroit, which will provide them with enlarged facilities for taking care of this constantly growing business.

BOOK REVIEWS

AIDS TO PHARMACEUTICAL LATIN, by G. E. Frease. William Wood & Co., New York, publishers. Cloth bound. 168 pages. $1.50.

A well-written, neat book designed for students in pharmacy and medicine. The author has included a very good Latin-English vocabulary and the text includes many exercises and examples.

The work is offered in a way which is clear and understandable, with the possible exception of the third declension of nouns, which might have been grouped in a better way, by which the student might more easily learn the gender of the different words of this declension.

The value of the book might also be increased by the addition of an English-Latin vocabulary.

R. L. C.

A TEXT BOOK OF PHARMACEUTICAL ARITHMETIC, by Theodore J. Bradley. Published by Lea & Febiger, Philadelphia, Pa. 12mo. Cloth bound. 196 pages. $2.25.

The author has omitted almost entirely any mention of the theory of arithmetic and the book consists of arithmetical problems designed to carry the student through a course in collegiate pharmacy; preparing him for State board examinations, and also may be used by the practising pharmacist in the manufacturing laboratory or the dispensing room.

The context begins with Ratio and Proportion, and in order takes up the various systems of Weights and Measures and their relationship to each other; then follows: Thermometric Scales, Calculation of Doses, Reducing and Enlarging Recipes, Specific Gravity, Stock Solutions, Percentage Dilution and Concentration, Alligation, and concludes with a chapter on Chemical Problems.

The book contains a very large number of problems and examples and is quite free from errors for a first edition. It is admirably suited for college work.

R. L. C.

MATERIA MEDICA AND THERAPEUTICS, INCLUDING PHARMACY AND PHARMACOLOGY, by Reynold Webb Wilcox. Published by P. Blakiston's Son & Co. 12th edition.

In June, 1926, we published a review of the eleventh edition of this popular work. The changes made in the twelfth edition, outside of the size of the pages and the preface, seem insignificant. The title page, as also the preface, would lead one to suppose that Dr. Wilcox had at length accorded to the National Formulary the recognition which its legal status demands; but an examination of the text reveals that he has not. The almost complete disregard of the National Formulary greatly lessens the value of the book as a work of reference to the pharmacist or as a textbook for the student of pharmacy.

H. C. WOOD, JR.

THE HISTORY OF PHARMACY IN STEIERMARK (AUSTRIA) UP TO 1850, by Dr. Norbert Schniderschitsch. Published by the Society for the History of Pharmacy. 140 pages. 10 marks.

The author discusses in eighteen chapters, instructively and entertainingly, the historical development of pharmacy and thus a phase of the cultural development of one sector of Austria. We learn that pharmacy was first definitely separated from medicine in Italy in the thirteenth century, somewhat later in Germany, and, according to certain records, in Steiermark in the sixteenth century.

The findings are evidently based on a thorough search (sources are given) and one observation from every chapter is here selected.

1. The county first owned the pharmacy and rented it, later it sold the pharmacy with definite exclusive privilege to appointed "county pharmacists," supporting them partially for many years.
2. Court pharmacies were rather generally attached to, and wholly or partly supported, until finally the titles "court-pharmacy" and "court pharmacists" only were conferred

without any special contribution from the court in many cases.

3. Inspections which were first inadequately carried out by physicians, although they lasted often several days, were more successful, when accomplished under Maria Theresa's reign by commissions of physicians, pharmacists and professors of natural science, who inspected pharmacies yearly for a few hours.
4. A uniform price schedule, in spite of many earlier attempts, was only made effective in 1756, through general adoption of the Vienna schedule for the official and through the adoption of other schedules for non-official drugs and their enforcement over seventy years later through Maria Theresa's government.
5. For the purpose of uniform preparation of drugs the Pharmacopœia Augustana was made official in 1657, the Augsburg Pharmacopœia in 1660, the Vienna one in 1737, the Austrian Pharmacopœia in 1774.
6. Instructions for pharmacists were announced from time to time, so that no prescription should be accepted for compounding without the signature of the physician, and this instruction was made binding through a rule announced in 1770.
7. From a mere prolonged experience training, a university course in chemistry and botany was made obligatory in 1811, followed by a special examination before the medical faculty. An apprentice had to collect at least 300 plants before advancement.
8. Female pharmacists were only found in monasteries. While probably first introduced by Italians, pharmacy was greatly furthered in its subsequent development by Germans.
9. The establishment of pharmacies remained a restricted privilege. One pharmacy, for 3000 to 4000 people, was declared sufficient in an 1823 court decree, although more people were usually served by one pharmacy.
10. As everywhere else pharmacists were confronted with competition here from monks, barbers, distillers, surgeons, patent medicine peddlers. This competition was ultimately eliminated. The druggist competition, however, grew until a

mutual agreement regulated the field of activity, the pharmacies agreeing to restrict their sales in confectionery and spices in exchange for a greater monopoly in retail drug trade.

11. The business fluctuated through the centuries and with it the wealth of pharmacists; however, in the time around 1667 many of the village pharmacists were forced to carry on other work such as farming for support.
12. The social position of the pharmacist was in general not quite that of the physician, though the court pharmacists ranked as officers of the army.
13. In 1830 the first general meeting of an organized pharmacists' association was held expressing as its aim the betterment of professional interests and of pharmacy as a whole.
14. In the contacts between pharmacists and physicians we find interesting developments; so in 1580 that the pharmacist is forbidden to direct a patient to a specific physician, in 1829 that a pharmacist is forbidden to send the physician even insignificant gifts such as confectionery or tobacco.
15. In addition to crude drugs, balsams and pills, we find in early reports also spices, inks and paints in the stores of pharmacies.
16. Inspecting physicians pointed out again and again in their reports that, contrary to orders, the medicine mixtures "compositæ," were mixed in their absence.
17. Only in 1837 the pharmacists were freed from the obligations to furnish medicines to paupers without compensation; voluntarily the pharmacists granted the communities a 50 per cent. reduction in the cost of medicines.
18. The difficulty of obtaining the help of pharmacists in military emergencies to the degree desired, ultimately led to the regular appointment of military pharmacists.

ARNO VIEHOEVER.